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A Glacial Acetic Acid-soluble Protein and a Hexosamine-containing Polysaccharide from a Liver-metastasis of Gastric Cancer.*

By

Yasuaki Matuda.

(松田 靖皓)

(From the Medico-chemical Institute (Director: Prof. H. Masamune) and the Medical Clinic of Prof. T. Kurokawa, Tohoku University, Sendai.)

(Received for publication, June 1, 1950)

Defatted residue of a liver-metastasis of gastric cancer[1] was subjected to our usual procedure of fractionation (Cf. Satoh[2] and Oh-Uti[3] and others) and a carbohydrate and a glacial acetic acid-soluble protein separated. The carbohydrate was revealed to be identical with the blood-group carbohydrate in gastric cancer[3] from its composition, optical rotation and blood-group activity. The protein differed from the corresponding fraction (a glucidamin) of gastric cancer because it contained too little a carbohydrate to be accepted as a glucidamin and was enormously levorotatory. Parcicularly worthy of note was its definciency of blood group activity and of giving Matsubara[4] cutaneous reaction of cancer patients. Paper chromatographic spots (Detailed information will be given elsewhere) suggested the presence in it of glucosamine and mannose in addition to chondrosamine, galactose and a methylpentose which were constituents common to it and the mentioned glucidamin.

From what have been described the writer inclines to believe that, generally, metastases of cancer are only partially identical regarding their high-molecular components with the original growth (Cf. also Aikawa and Siozima[5]). Nevertheless, on the other hand, the components of the metastasis above beared no resemblance to the corresponding components of liver in which the metastasis occurred (Cf. the foregoing article[6]). Here the discussion is confined to the components of parenchymal cells.[7][8]

Experimental.

Aikawa[1] placed in cold alcohol a large liver-metastasis of gastric cancer (cut into pieces) from a Group B person and then boiled a part

* 137th report of Masamune and co-workers' "Biochemical Studies on Carbohydrates."

of thus dehydrated material with alcohol for isolating the blood group lipoid. The remaining dry material was similarly defatted by the writer and together with the residue Aikawa left (255 g. altogether) extracted with five 2 *l.* portions of water, each time shaking on an apparatus for 2 days (preservative, toluene). The fifth extract gave no Molisch reaction. Others were distilled in vacuo to a syrup and 5 volumes of alcohol added. The precipitates, when dry, amounted to 5.5 g. 2.4 g. of the powder (yellow colored) were extracted with water many times and after centrifuging off the insoluble part, freed from the substance precipitable with baryta at pH 9.0 of the solution and the one precipitable by acidulation with acetic acid (The latter was little in quantity), as in the experiment accounted for in the foregoing report[6]. After treatment with sufficient H_2SO_4 to remove barium, dialysis, condensation and addition of alcohol by turns, 1.3 g. precipitates as dried were obtained. The present substance was divided into glacial acetic acid-soluble and -insoluble fractions, by kneading with a few drops of glycol and precipitating with glacial acetic acid (5 cc. were added in portions). The deposit was washed with glacial acetic acid (4 changes) and with ether and dried (non-colored powder; 0.2 g.) and further the portion soluble in 5% trichloracetic acid was separated, by extracting with one 10 cc. and one 5 cc. portion of the trichloracetic acid solution and precipitating with alcohol—Crude Polysaccharide (110 mg.). To the mother liquid of the glacial acetic acid-precipitates above and the washings of the latter were added 3 volumes of ether, and again the deposit here was washed with ether and dried—Crude Protein (1.1 g.).

The crude polysaccharide did not contain phosphorus (ammonium molybdate test with oxidation) and glycogen (iodine test). It gave distinct Molisch reaction but was also biuret positive. N (micro Kjeldahl) 8.0%, hexosamine 20.8%, non-amino sugars as galactose 21.1% (The indole, orcinol, α-naphthol (I) and diphenylamine (I) reaction gave sugar values pretty coincident as expressed as galactose. 21.1% was the average), ash 2.0%.

Isolation of the Polysaccharide. 75 mg. of the crude polysaccharide were dissolved in 0.7 cc. water, and after addition of 9.03 cc. of 98% glacial acetic acid, shaken for 3 days. Centrifuged. The centrifugate was washed with 5 cc. of 90% acetic acid, with acetone and with ether and dried—50 mg. The amount of non-amino sugars was raised to 35% as galactose. The figure was the mean of those, almost identical, obtained by the four colour reactions above. 1 mg. of the product was taken for this analysis.

Further purification was tried by the copper-alkali procedure: The balance of the substance was dissolved in 3 cc. of water and 1 cc. of 0.5 m. $CuSO_4$ solution added, then the mixture was alkalized with 10% NaOH to a pH$>$10 and the precipitates centrifuged. The centrifugate was

stirred up in 10 cc. of water, and after addition of a small quantity of dilute NaOH, again agitated and centrifuged. The centrifugate here was suspended in 5 cc. of water and dissolved with just sufficient 10% H_2SO_4 and precipitated over again with alkali. The operations of washing of the precipitates with alkali, dissolution and precipitation were repeated as long as the biuret-giving substance remained and finally the centrifuged precipitates taken up in a smallest possible quantity of 10% HCl. To the solution obtained were added 5 volumes of absolute alcohol and the deposit was separated, kneaded to a paste with a few drops of 1% HCl and dehydrated with alcohol. After thorough washing with plain alcohol it was dried. There were yielded 32 mg. of non-colored powder. It was difficultly soluble in water.

Analysis of the product: N(micro Kjeldahl) 4.7%, hexosamine 37.8%, non-amino sugars as galactose 38% (The indole, orcinol and α-naphthol (I) reaction gave agreeing figures of galactose), ash (micro method) 4.6%. Optical rotation:— 14.5 mg. substance was dissolved in 0.5 cc. of 0.01 n. NaOH and filled up to 1 cc. with water. The pH of the solution was 8.6. $[\alpha]_D^{12.5}=\frac{-0.56\times 100}{14.5\times 1}=-38.6°$ ($-40.5°$ on ash-free basis).

It exhibited Group B specific activity (inhibition of isohemoagglutination) at a dilution of 1:8000.

Isolation of the Protein. As when the glucidamin of human liver was purified,[8] the crude protein was treated with baryta and HCl. The product thus freed from phosphorus (gray powder; 0.4 g.), was soluble in water and hardly precipitated with $(NH_4)_2SO_4$. It was not cast down by trichloracetic acid added to 5% but was by this precipitant at 10% concentration. It showed the positive biuret and Molisch reaction. Pentose, methylpentose and cholesterol were not detected. A trace of hydrolysable sulfur was present. N(micro Kjeldahl) 15.4%. Hexosamine 2.7%. Non-amino sugars as galactose (Values almost coincident, 3.0, 3.0 and 2.9%, were obtained by the indole, orcinol and α-naphthol (I) reaction) 3.0%. Ash 0.64%. $[\alpha]_D^{12.5}$(in water)$=\frac{-14.3\times 100}{1\times 1.7}=-84.1°$. Inhibition of isoagglutination of erythrocytes was not shown nor was given Matsubara cutaneous reaction in patients with cancer.

The product was further subjected to electrodialysis, but the substance, that had been recovered from the solution by addition of 5 volumes of alcohol and a little anhydrous sodium acetate, analyzed similar to above.

Summary.

From a liver-metastasis of gastric cancer were isolated a group carbohydrate and a protein. The former was found to be identical with the

one obtainable from gastric cancer and the latter to differ not only from the corresponding fraction of gastric cancer but also from the corresponding one of normal human liver.

Through the Grant Committee of the Science Council was given a grant from the Education Department, which enabled us to carry out this work.

H. Masamune.

References.

1) Aikawa, Tohoku J. Exp. Med., 1949, **51**, 367.
2) Satoh, T., ibid., 1949, **51**, 275.
3) Oh-Uti, K., ibid., 1949, **51**, 297.
4) Matsubara, Tokyo Iji Shinshi, 1950, **67**, No. 5, 17.
5) Siozima, Tohoku J. Exp. Med., in press.
6) Matuda, ibid., 1950-51, **53**, 383.
7) Masukawa, ibid., in press.
8) Siozima and Masukawa, ibid., in press.

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Biochemical Studies on Carbohydrates.

CXXXVIII. A Search for the Unknown Nitrogenous Determinant Group of Masamune in the Group Carbohydrate from the Mucus-mucin of Pig Stomach.

By

Yasuaki Matuda and **Eitaroh Itoh.**
(松田順皓)　(伊藤榮太郎)

(From the Medico-chemical Institute, Tohoku University, Sendai. Director: Prof. H. Masamune.)

(Received for publication, June 1, 1950)

Masamune[1] claimed that group (ABO-system) carbohydrates possess two kinds of determinant group, one a structure formed by either glucosamine or chondrosamine together with either galactose or galacturonic acid or D-fucose and the other an unknown nitrogenous structure or constituent. As to the components of group lipoids one[2] of the writers has fractioned the hydrolysate of a preparation of the group lipoid from A human liver and isolated choline and arginine besides glucosamine, galactose and others. Whether or not choline and arginine were real constituents of that lipoid is not certain at present, but it will be worthy of experimentation for the scrutiny of the second determinant group to decide if preparations of group carbohydrates involve phosphotungstic acid-precipitable components particularly choline and allied quaternary methylamine derivatives such as neurine and glycocollbetaine. In the present work the writers picked out Group A carbohydrate from the mucus of pig stomach as a material and searched after the compounds in question among the split products. The hydrolysis was exerted in two steps, first with 1 n. H_2SO_4 and next with 6 n. HCl. Choline and neurine which are cast down from alcohol by platinum chloride, glycocollbetaine whose hydrochloride is insoluble in alcohol and which is precipitated from water with gold chloride, histidine and lysine were not found, whereas arginine was isolated as flavianate. Arginine, however, was yielded in too small an amount to be accepted as a constituent of that carbohydrate.

Experimental.

The group carbohydrate was prepared immediately from the mucus by our usual method (Cf. f. i. Yosizawa[3]) Electrodialysis was not effected. The copper alkali procedure was carried out as described by Matuda.[4]

The product analyzed: nitrogen 4.3%, glucosamine 40.1%, galactose 41.6%, ash 2.1%. The inhibition of isoagglutination of A erythrocytes was exhibited at its dilution of $1:16^6$. Negative biuret despite of positive Sakaguchi test for arginine. Tollens ammoniacal silver solution was reduced but very slowly. Positive reduced sulfur test and Acree-Rosenheim test for tryptophane were not given.

1) *Primary Hydrolysis of the Group Substance and Fractionation with Phosphotungstic Acid.* 5 g. of the material were taken up in 250 cc. of 1 n. H_2SO_4 and refluxed for 5 hours at 100°C. When cold, the hydrolysate was filtered and to the filtrate was added 20% phosphotungstic acid in portions until no more precipitation occurred. After standing for 5 hours the deposit was centrifuged and washed with 1 n. H_2SO_4 containing 2.5% phosphotungstic acid. It was then suspended in 30 cc. water and freed from phosphotungstic and sulfuric acid with the saturated $Ba(OH)_2$. The solution obtained had 41.5 mg. nitrogen.

2) *Fractionation at Acid Reaction of the Phosphotungstic Acid-precipitable Substance with Silver Sulfate.* The final solution above was distilled under diminished pressure to 25 cc. and after centrifuging off of some appearing deposit, dilute H_2SO_4 and fine powdered Ag_2SO_4 added whereby precipitates were given. The centrifuged supernatant liquid and the centrifugate suspended in water were separately freed from silver and sulfuric acid by the aid of H_2S and the saturated $Ba(OH)_2$, and the fluid containing the substance, that had been precipitated with Ag_2SO_4, was further condensed to a small volume (about 1 cc.). The condensate (neutral and somewhat turbid) gave neither murexide reaction with halogen nor diazo reaction with NaOH, whereas its dried up residue showed the positive Lassaigne reaction of nitrogen, suggesting the presence of guanine. The other fluid containing the fraction unprecipitated by Ag_2SO_4 were assayed qualitatively with following results: ninhydrin+, biuret+, arginine (Sakaguchi)+, histidine (Totani, Hanke-Koessler)− ?, tyrosine (Totani, Hanke-Koessler) −?.

3) *Subdivision with Alcohol of the Fraction Unprecipitated by Silver Sulfate.* The balance of the fluid concerned was acidified to pH 1.0 with HCl and distilled in vacuo to a small volume. This was subjected repeatedly to vacuum distillation with water or with alcohol and when excess HCl was eliminated, the residue was dissolved in 2 cc. of water and 20 cc. of absolute alcohol added, whereupon brownish-colored precipitates came out. The centrifuged supernatant fluid was distilled to dryness and absolute alcohol added. A part of the substance did not dissolve. Centrifugation and vacuum distillation of the supernatant liquid and addition of absolute alcohol to the remainder was repeated until all the alcohol-insoluble part was separated. The final alcoholic solution gave no precipitates with

alcoholic platinum chloride solution (2%) which indicated that no choline nor neurine was present, because the hydrochlorides of these quaternary amines are soluble in absolute alcohol while that of glycocollbetaine is not. The balance of the solution was evaporated similar to above and the residual taken up in 10 cc. of water. A small insoluble part was centrifuged and set aside. Precipitation tests on the clear solution: It gave 1) precipitates with phosphotungstic acid (A part of the solution was acidified with H_2SO_4 and the precipitant added), and also 2) some precipitates with picric acid as dissolved in water and with flavianic acid as dissolved in water (The presence of a small amount of arginine was suspected) but 3) no precipitation with watery platinum chloride solution (2%) (Neurine was scarce, if present).

4) *Extensive Hydrolysis of the Balance of the Fraction Examined in* 3) *with Following Fractionation with Phosphotungstic Acid.* The alcohol-insoluble portions, the water-insoluble substance and the watery solution above were combined and distilled in vacuo to dryness. The residual was hydrolysed with 20 cc. of 6 n. HCl by heating for 16 hours in a boiling water-bath (under return condenser). The hydrolysate was diluted six times with water and filtered and the filtrate (biuret-negative) precipitated with phosphotungstic acid with following removal of the precipitant from the precipitates in a similar manner as in 1). The solution obtained was made up to 100 cc. for determination of nitrogen. 30.8 mg. N was present. Then 1 cc. of 10% HCl was added and the mixture distilled at a low temperature to about 2 cc. Addition of 10 cc. of alcohol gave rise to mere turbidity but no precipitation. The turbid fluid was distilled as such to dryness. Excess HCl was eliminated by repeated vacuum distillation of the residue with water and with alcohol, discarding a very small amount of water-insoluble substance in the course of the operations. And the final evaporated residue was taken up in 4 cc. of water. The solution here (pH, 5.0) was found non-precipitable by watery gold chloride solution (2%) (glycocollbetaine —). A few drops of the solution were taken for that test. Again the balance of the solution was distilled. The dried-up remainder was taken up in 20 cc. of absolute alcohol. The insoluble part centrifuged and then the supernatant liquid distilled to dryness. From the remainder the alcohol-insoluble part was further separated in a similar manner and this time the solution was condensed to about 5 cc. A few drops of the solution were put to the test with the alcoholic platinum chloride solution but no precipitation happened (choline —, neurine —).

5) *Fractionation with Silver Nitrate at Alkaline Reaction of the Substance Precipitated with Phosphotungstic Acid in* 4). The remaining portion of the alcoholic solution was distilled and the residual thereby and the substance undissolved in alcohol were dissolved together in 15 cc. water. The solu-

tion was acidified with HNO_3 and sufficient 20% $AgNO_3$ added for the most possible precipitation. The deposit was centrifuged[5], and to the supernatant liquid was further added the $AgNO_3$ solution in small portions until a drop of the mixture gave brown precipitates on bringing together with a drop of the saturated $Ba(OH)_2$, and then at first neutralized with the saturated $Ba(OH)_2$ and afterwards saturated with powdered baryta. The dark precipitates were centrifuged and washed with changes of diltue $Ba(OH)_2$ with centrifugation. The supernatant fluids were soon freed from barium and silver with H_2SO_4 and H_2S, subsequently distilled in vacuo to about 5 cc., and after addition of an equal volume of 10% H_2SO_4, precipitated with 20% phosphotungstic acid. The precipitates obtained (very few) were suspended in water and liberated from phosphotungstic acid with a mixture of amylalcohol and ether (1:1) and from H_2SO_4 with a just sufficient quantity of the saturated $Ba(OH)_2$ and the resultant clear solution was condensed to about 0.5 cc. and diluted with 2 cc. of absolute alcohol. Addition of saturated alcoholic picric acid solution caused no precipitation (lysine —). The mixture was diluted with 20 cc. of water, acidified with HCl and shaken with ether. And the picric acid-free solution was distilled in vacuo to dryness and to the little rest were added 2 cc. of absolute alcohol. However, all the substance dissolved thereby, which proved the absence of glycocollbetaine. Really precipitation test on drops of the solution with alcoholic platinum chloride solution resulted in negative. Further, the balance of the solution was distilled to dryness and the remainder dissolved in 1 cc. of water (All the substance dissolved). This solution, too, gave no precipitate with watery gold chloride solution (2%).

6) *Isolation of Arginine.* To the dark Ag-precipitates in the preceding section was added dilute H_2SO_4 in excess and the centrifuged supernatant liquid bubbled with H_2S. Again centrifuged. Ag_2S (the centrifugate) was washed with boiling water and filtered. Washing was repeated. The supernatant liquid and washings were boiled together to expel off H_2S. (Diazo test for histidine was negative at this stage). The excess H_2SO_4 was removed with an equivalent quantity of the saturated $Ba(OH)_2$. And the solution was distilled in vacuo to about 1 cc., 5 cc. of 5% flavianic acid (a watery sol.) added and the mixture stood for 2 days. Yellow crystalline deposits (octahedrons) were collected (on a suction funnel) and dried at 105°C — 24.2 mg. The product was recrystallized from a hot very dilute solution of flavianic acid and dried as above — 20.2 mg. N (Friedrich), 17.12% (calculated for arginine flavianate, 17.21%). It began charring at 258°C and decomposed at 268°C.

7) *Examination of the Filtrate from the Crude Arginine Flavianate.* This filtrate was freed from flavianic acid by addition of the saturated $Ba(OH)_2$.

After quantitative removal of excess barium with H_2SO_4, the solution was distilled to about 5 cc., when its pH lay at 4.5. The condensate gave no positive ninhydrin reaction.

Conclusion.

Group A carbohydrate from the mucus-mucin of pig stomach contains neither choline nor neurine nor glycocollbetaine nor histidine nor lysine. From the hydrolysis products of this group substance arginine was isolated as flavianate but the yield was too small for a constituent.

We express gratitude to the Education Department which gave a grant through the Grant Committee of the Science Council in aid to us.

H. Masamune.

References and Notes.

1) Masamune, Tokyo Iji Shinshi, 1949, **66**, 269. Cf. also Matuda (2)).
2) Matuda, Tohoku J. Exp. Med., 1951, **53**, 373.
3) Yosizawa, ibid., 1949, **51**, 51.
4) Matuda, ibid., this volume, p. 1.
5) The centrifugate was suspended in water, and after removing Ag with H_2S, the solution was distilled but nothing left.

A Method to Take a Kymogram of the Heart along the Perimeter of the Transsection.*

A Preliminary Report.

By

Shinji Takahashi and **Tatsuyo Shinozaki.**
(高橋 信次) (篠崎 達世)

(From the Department of Radiology, Hirosaki University School of Medicine. Hirosaki)

(Received for publication, March 28, 1951)

An X-ray tube, a rotation table, a lead slit and a film cassette are arranged in this order for the operation.

The film moves just behind the stationary slit which is 1 mm wide,

* Read at the 3rd Meeting of the Tohoku Radiological Society. Oct. 15, 1948. Sendai.

and out cut horizontally in the lead plate. A lead wire is set across the slit where it is intersected by the plane, containing the tubefocus and the rotationaxis of the rotationtable. And on the rotationtable a patient is placed.

1) Discontinuous Rotatokymography:

Rotating the rotationtable from 0° to 180°, the heart of the patient is exposed at every 10° of rotation discontinuously under the following conditions: Focus-rotationtable-distance 75 cm, Rotationtable-film distance 25 cm, 80–85 KV., 40–45 mA., 2 seconds. Thus we get 19 stripes of one-slit kymograms of the heart taken from different projections.

Each stripe records the movement of the heart at each point in the perimeter of the transsection. At the center of the film there is imaged a lead wire line which represents the rotationaxis of the rotationtable and is called a standard line.

On a drawing table we take three points A, B and C. The distance between them is made the same as that of the tube focus, the rotationaxis of the rotationtable and the film. A drawing paper is fixed with a nail at the point B. When we adjust the standard line of the kymogram taken at 0° projection to the point C and join with a pencil the point A with the crest and the trough of the wave of both sides in the X-ray image of the heart, we have four lines drawn on the paper. Rotating the paper around the center B every 10° discontinuously, we repeat this process with each kymogram. Then we get two concentric, curved perimeter.

These figures represent the transsections of the heart in actual size at its systole and diastole.

2) Continuous Rotatokymography:

Rotating the rotation table from 0° to 180° continuously and moving the film synchronously to it, we expose under the following conditions: 80 KV., 30 mA., 20 seconds.

The film thus taken has a series of images showing the movement at every point of the transsection of the heart. The analysis of this continuous rotatokymography is performed after the law of the continuous rotatography.

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Modulators and Dominators of the Toad's Retina.

By

Susumu Tukahara.
(塚原　進)

(Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai)

(Received for publication, June 10, 1950)

Granit's[1][2][3][4] extensive work on the spectral sensitivity of retinal elements isolated by the micro-electrode technique shows that there can be distinguished three kinds of retinal elements which show relatively narrow distribution curves of sensitivity with maxima around 600, 530 and 460 mμ and two kinds of elements which have broader curves with maxima around 500 and 560 mμ. Granit called the retinal elements showing narrower bands "modulators" and those having broader bands "dominators".

In a previous experiment Motokawa, Iwama and Tukahara[5] analyzed retinal processes of the toad's retina by Motokawa's method, taking spikes from retinal elements isolated with the aid of the micro-electrode technique as the index of excitation, and found that there are three groups of retinal elements which can be distinguished by the Motokawa's method. They inferred that the three kinds of elements are to be identified with the three kinds of modulators distinguished by the Granit's method, but the identification is not complete, because no examination of the spectral sensitivity of the elements was carried out in their experiment. I have, therefore, undertaken in the present investigation to examine the electrical excitability and spectral sensitivity of one and the same retinal elements in order to confirm the validity of the claim made by Motokawa *et al.*

Method and Procedure

The experimental arrangements used in the present experiment are shown in Fig. 1. Discharges from a single element or a limited number of elements were picked up by means of a micro-elecrode, M which was inserted into a toad's retina, R after removal of the cornea and the lens. The discharges were observed on a screen of a cathode ray oscillograph, and at the same time listened to in a loudspeaker. Light from various parts of a glass spectrum, Sp was projected onto the retina by means of a rectangular prism, RP and a concave mirror, CM. The wave-lengths of spectral lights were calibrated in comparison with line spectra from a

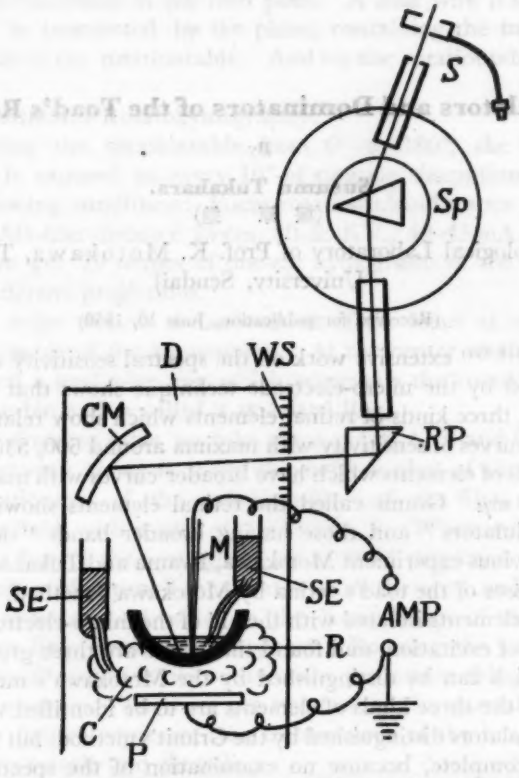

Fig. 1. Experimental arrangement. AMP: amplifier (R-C type, four stages). C: Ringer-soaked cotton. CM: concave mirror. D: box with dark walls. M: micro-electrode. P: silver plate. R: retina. RP: rectangular prism. S: shutter. SE: stimulating electrode of Zn-$ZnSO_4$-gelatine type. Sp: spectroscope. WS: scale for reading wave-lengths.

sodium and a cadmium lamp, and could be read on a scale, WS. The light source for the spectroscope was an automobile head light lamp with a known distribution of spectral energy.

In the first place, a distribution curve of spectral sensitivity of an element was determined in the same manner as in the Granit's experiments and then the eye was dark-adapted for about 10 minutes in order to make measurements of electrical excitability. For electrical stimulation of the retina, a pair of non-polarizable electrodes of Zn-$ZnSO_4$-gelatine type was used. The stimulating current used was a single constant current pulse of one second in duration with the cathode inside the retina, the index

being a make-response.

Measurements were carried out in the following manner: The eye was exposed to fairly strong white light (not to any spectral light) for two seconds, and after cessation of the illumination the change in electrical excitability was measured at constant intervals of 30 seconds till the excitability became constant. This constant level was chosen as the reference level of excitability. The increase in electrical excitability above this reference level was expressed in percentage of the excitability at this level and plotted against time after cessation of the pre-illumination. Curves so obtained will be called "excitability curves."

Since it was difficult to repeat many times such a long series of measurements on one element, I endeavored to determine an excitability curve from data obtained in a single or at most two series of measurements. For this purpose, it was tried to adjust the intensity of each electrical stimulus to the threshold intensity at each moment, although the trial failed very often. Such adjustments may appear to be very difficult, but in reality for trained experimenters it was rather easy because they could foresee the time course of the excitability curve to be constructed from the data already obtained from the measurements of spectral sensitivity of the element in question.

I have so far succeeded in dertermining spectral distribution curves of photosensitivity for 71 elements, but measurements of electrical excitability were successful only on 25 elements of them; the rest had to be discarded owing to sudden decreases in excitability during electrical measurements.

Results.

1. *Influence of Temperature on the Excitability Curves.*

The excitability curve obtained from the intact eye by pre-illuminating with white light shows generally three distinct humps R, G and B at about 1.5, 3 and 5 minutes at room temperature around 20°C, as shown by Motokawa[6]. In the present experiment, however, the crest times of the three humps were found much longer. This is obviously due to the lower temperature at which the present investigation was carried out. Fig. 2 serves to illustrate this influence of temperature on the time course of the excitability curve. The two curves in the figure were obtained at two different temperatures 8° and 18°C, by taking the action potentials from the whole optic nerve as the index.

The upper curve was constructed from the data obtained in a single series of measurements, while the lower represents the average of three series of measurements successively taken. The marks +, −, and ± used in the upper curve mean respectively a just perceptible response, no

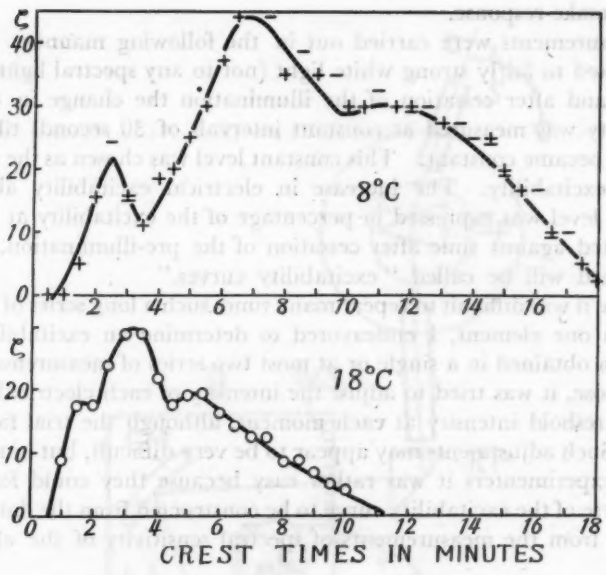

Fig. 2. Excitability curves for white light obtained from the whole retina with action potentials from the optic nerve as the index.

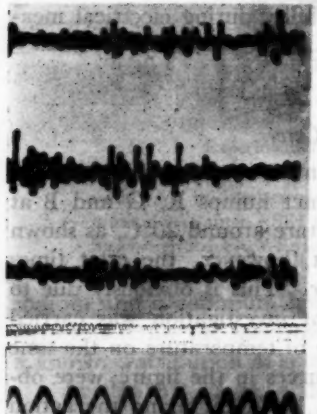

Fig. 3. Isolated spikes from three retinal elements in response to illumination. Time mark 1/200 sec.

response and a response perceived either on the screen of the cathode ray or in the loudspeaker. As can be seen in the figure, the crest times of the R, G and B elevations are about 2.5, 6.5 and 11 minutes respectively at temperature 8 °C. Such a low temperature was found rather favorable for this kind of analysis, because the time course of each elevation of the excitability curve can be determined more accurately than at higher temperatures.

2. *Excitability Curves of Modulators.*

In the following experiments the index of excitation was always isolated spikes from single or a limited number of elements. Some examples of such spikes in response to illumination are shown in Fig. 3. As mentioned above, modulators have been

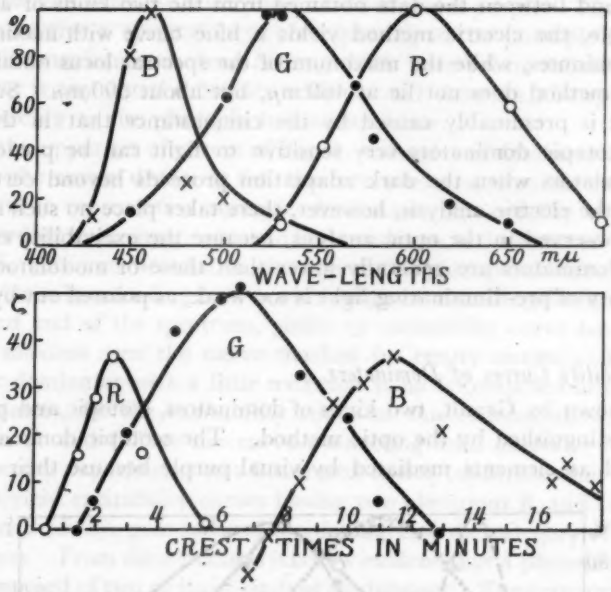

Fig. 4. Spectral distribution curves of photosensitivity for three kinds of modulators R, G and B (above) and excitability curves of one and the same elements (below). Curves with the same marks correspond to each other.

defined by Granit as retinal elements whose spectral locus of sensitivity comprises a relatively narrow band of the spectrum. Examples of the three kinds of modulators are illustrated in the upper diagram of Fig. 4. The red or R modulator has a maximum at about 600 mμ, the green or G modulator at about 530 mμ and the blue or B modulator at about 460 mμ. The excitability curves of the same retinal elements as illustrated in the upper set of curves are shown in the lower diagram of Fig. 4. The curves corresponding to each other are denoted by common marks. As can be seen in this figure, the excitability curve of the R modulator has a crest time of about 2.5 minutes, that of the G a crest time of about 6.5 min. and that of the B a crest time of about 11.5 min. Such definite correspondence was found on all the retinal elements of the modulator type on which the two kinds of analysis, optic and electric have succeeded. Such data obtained from one and the same retinal elements provide us with the most convincing evidence that both optic and electric methods of analysis yield consistent results, so far as modulators are concerned.

It is, however, to be noted that in some cases apparent disagreement

can be found between the data obtained from the two kinds of analysis; for example, the electric method yields a blue curve with maximum at about 11 minutes, while the maximum of the spectral locus obtained by the optic method does not lie at 460 mμ, but about 500 mμ. Such disagreement is presumably caused by the circumstance that in the optic analysis scotopic dominators very sensitive to light can be predominant over modulators when the dark adaptation proceeds beyond certain extent. In the electric analysis, however, there takes place no such masking effect as observed in the optic analysis, because the excitability curves of scotopic dominators are generally lower than those of modulators unless the intensity of pre-illuminating light is too weak, as pointed out by Motokawa[6)7)].

3. *Excitability Curves of Dominators.*

As shown by Granit, two kinds of dominators, scotopic and photopic can be distinguished by the optic method. The scotopic dominators are considered as elements mediated by visual purple because their spectral

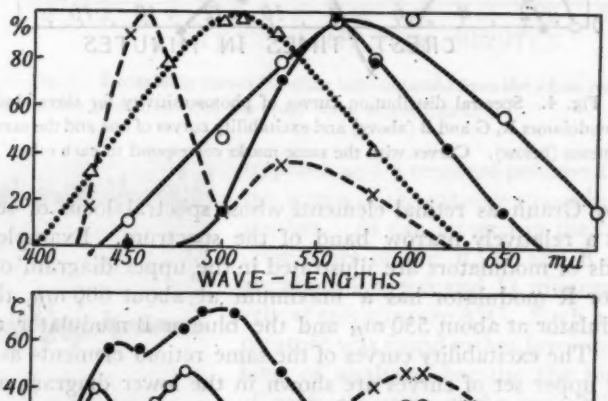

Fig. 5. Upper set of curves: spectral distribution curves of photosensitivity for two photopic dominators (full lines), a scotopic dominator (dotted line) and curves composed of B and G modulators (broken line). Lower set of curves: excitability curves each corresponding to above distribution curves with the same marks.

locus coincides with the absorption curve of visual purple. The photopic dominators give a spectral locus corresponding to the photopic luminosity curve with maximum at about 560 mμ. We have yet no reliable evidence that there is any visual substance which would show an absorption curve corresponding to the photopic luminosity curve.

In the upper set of curves of Fig. 5, examples of dominators are illustrated; the curves drawn in full line are photopic dominators, and the dotted curve represents a scotopic dominator. Excitability curves each corresponding to the upper spectral loci are shown in the lower set of curves of Fig. 5. As can be seen in the figure, the photopic dominator whose spectral locus is extending over a wide range of wave-lengths from 440 mμ to the red end of the spectrum, yields an excitability curve having three distinct maxima (see the curve marked by empty circles). The other photopic dominator with a little narrower band is composed of the R and G modulators, for the excitability curve of this element shows the two elevations R and G (see the curve connecting solid circles).

In many experiments not illustrated it was confirmed that such elements as yield excitability curves having two elevations R and G or three R, G and B always give spectral loci falling into the category of photopic dominators. From these findings it is now evident that a photopic dominator is composed of two or three kinds of modulators. The composite nature of photopic dominators was suggested by Granit[9] on the basis of his experiment on selective adaptation. When the photosensitivity of any of these components of a photopic dominator is decreased as the result of selective adaptation, the composite nature of the dominator can be disclosed even by the optic method of analysis. In the electric analysis each component of a dominator is clearly seen as a distinct hump on the excitability curve without any special procedure such as selective adaptation. It seems therefore that the electric method of analysis has a higher resolving power than does the optic method.

The way in which modulators are combined into a dominator is not always so harmonious as give a smooth single dominator curve as a whole, and in some cases each component appears separately in the spectral distribution curve. The broken curve in the upper diagram of Fig. 5 respresents such an example. In this case the curve consists of a green and a blue modulator.

For scotopic dominator no reliable data could be obtained as to their excitability curves probably because this kind of dominator is liable to masking effects which will be explained later.

Discussion.

In the above experiments the total of the elements analyzed by the

TABLE I.

Kinds of retinal elements	Number of elements	In percentage
Photopic dominators	26	36.6
Scotopic dominator	8	11.3
R-modulators	12	16.9
G-modulators	14	19.7
B-modulators	4	5.6
G-B-modulators	5	7.0
R-G-modulators	1	1.4
R-G-B-modulators	1	1.4

optic method amounted to 71, and the items are as shown in Table I.

As can be seen in this table the three kinds of modulators are markedly different in number. Especially, the number of the B modulators is so small that uneven probability for appearance of the three kinds of modulators is obvious although we have a small statistical sample before us. This finding hardly conforms to the result obtained by Motokawa *et al*[5] by the electric method of analysis. In their analysis the three kinds of modulators appeared in about the same number, what conforms also to the fundamental assumption of the trichromatic theory. The rare appearance of the B modulators cannot be attributed to chance, for this kind of modulators was rarely encountered also in the Granit's experiment on frog eyes[2].

The discrepancy must be due to the difference in the mode of analysis. As mentioned above, the B modulators are apt to be masked by scotopic dominators in the optic analysis, whereas the electric method suffers no such masking effect. And, this must be a merit of the electric method at least for the analysis of modulators.

The question now arises as to why the scotopic dominator cannot be detected so easily in the electric analysis as in the optic even when measurements are performed under complete dark adaptation. The answer to this question seems to be found in the structual difference of scotopic dominators and the other kinds of elements. The site of attack of electrical stimulation of the retina must be synapses in the retina; fibers of the optic nerve cannot show such striking supernormality following illumination as revealed by electrical stimulation. The fact that a pre-illumination decreases obviously the photosensitivity of photoreceptors in the retina, but increases the electrical excitability, rules out the possibility that electrical stimulation acts directly upon the photoreceptors themselves. As Granit[9] suggests, photopic dominators are retinal elements having the most elaborate synaptic organization, while there are many reasons to suppose that scoptoic dominators have much simpler synaptic organization. As one of the rea-

sons it was emphasized by Granit that pre-excitatory inhibition does not exist in the on-elements which yield scotopic dominator curves. It is now obvious that the electrical supernormality following illumination is a phenomenon at the synaptic level of the retina, and that the phenomenon is concerned with the pre-excitatory inhibition, because the phenomenon under consideration can be regarded as a kind of off-effect. If scotopic dominators are represented by pure on-elements, it is expected that scotopic dominators show only weak supernormality if any in our electrical measurements following a pre-illumination. This must be the reason why our electrical analysis of modulators is little disturbed by the presence of scotopic dominators.

Summary.

Retinal processes of the toad's retina were analyzed by taking spikes from single retinal elements or a restricted number of element as the index.

1. Curves of spectral distribution of photosensitivity for 71 retinal elements were mapped by Granit's optic method of analysis. 26 elements out of 71 were so-called photopic dominators, 8 scotopic dominators, and the rest so-called modulators.

2. It was shown that the modulators fell into three groups with maxima at three preferential regions of the spectrum. However, the number of elements belonging to the " blue " or B group was found much smaller than those of the other kinds of elements. This is due to the masking effect exerted by scotopic dominators upon the B group.

3. On 25 elements out of 71 Motokawa's electrical analysis succeeded. Three kinds of modulators show supernormal electrical excitability following an illumination by white light for two seconds, the time courses of excitability changes being different according to the kind of modulators.

4. It was shown very clearly by the electric method that photopic dominators are composed of two or three kinds of modulators. All these experiments indicate that the two methods of analysis, optic and electric yield consistent results, if the masking effect stated above is ruled out.

Thanks due to Prof. Motokawa for his good guidances and advices, and to Dr. Iwama for his co-operation.

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Der Einfluß des Atropins auf die Dünndarmbewegung.

Von

Takesi Hukuhara.

(福原 武)

(Aus dem Physiologischen Laboratorium der Medizinischen Fakultät zu Yonago.)

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Tatsache ist es, daß große Dosen von Atropin auf die Dünndarmbewegung hemmend einwirken. Was jedoch die wirkung der mittleren und kleinen Dosen von Atropin anbelangt, so gehen die Versuchsresultate der Autoren weit auseinander. Die Bewegung des ausgeschnittenen Dünndarmes wird nach Magnus[10][11]. Kress[7] und Baur[1] befördert, nach van Lidth de Jeude,[8] le Heux[3] und Hirz[4] durch Atropin allerdings im allgemeinen gehemmt, während sie nach Trendelenburg[13] und Liljestrand[9] bald fördernd bald hemmend beeinflußt wird. Außerdem sagen Unger[15] und Liljestrand[9], daß kleine Zugaben von Atropin auf die Darmbewegung hemmend und mittlere Zugaben fördernd einwirken. Am Dünndarm in situ bemerkten Trendelenburg[14] und Katsch[6] nur einen hemmenden Einfluß auf die Darmbewegung, während Hukuhara[5] ausschließlich eine befördernde Wirkung sah.

Die Ursache solcher Nichtübereinstimmung ist nach le Heux[3] auf den verschiedenen Gehalt des Cholins im Darm zurückzuführen. Am frisch ausgeschnittenen Darmpräparat, in dem Cholin reichlich enthalten ist, sieht man immer eine hemmende wirkung des Atropins, während man nach wiederholtem Auswaschen des Präparates mit Tyrodescher Lösung stets eine fördernde Wirkung desselben bemerkt. Gegen diese Ansicht le Heux's sprechen Baur[1] und van Esveld,[2] gestützt auf die Tatsache, daß sie auch am frischen Präparat immer eine fördernde Wirkung gesehen haben.

Aus den obenerwähnten ersieht man, daß die zahlreichen Widersprüche noch nicht gelöst sind. Neulich kam mir der Gedanke, daß diese Widersprüche höchstwahrscheinlich auf Mängel der bisher angewandten Untersuchungsmethoden zurückzuführen sind. Daß dies in der Tat der Fall ist, möchte ich im folgenden Versuchen zeigen.

Kritik und Wahl der Methoden.

Das Dünndarmrohr ist ein Hohlorgan, das aus der Längs- und Ringmuskulatur besteht. Wenn die Kontraktion der Längsmusklatur die der

Ringmuskulatur überwiegt, so verkürzt sich der Längsdurchmesser des Rohres, während sich der Querdurchmesser verlängert. Wenn sich dagegen die Ringmuskulatur stärker kontrahiert, so verlängert sich der Längsdurchmesser, wogegen sich der Querdurchmesser verkürzt. Was die Veränderung in der Weite des Darmrohres anbelangt, so haben beide Darmmuskelschichten entgegengesetzte Wirkungen. Wenn sich die Ringmuskulatur des Dünndarmes durch die Applikation eines Arzneimittels stärker als die Längsmuskulatur kontrahiert, so wird der Darm länger als zuvor und gleichzeitig bemerkt man eine Verkleinerung der Amplitude der Längenbewegungen. Wenn man dabei seine Aufmerksamkeit nur auf die Darmlänge lenkt, was bei der Magnusschen Methode der Fall ist, so würde man annehmen, daß die Darmbewegung gehemmt worden sei. Wie oben auseinandergesetzt, ist der Darm hierbei jedoch in Wirklichkeit nur in Erregung versetzt worden. Damit man sowohl die Erregung wie die Hemmung der Darmbewegung sicher feststellen kann, muß man also die Veränderung in der Kraft oder der Arbeit des Darmes untersuchen. Als Indikator der Kraft eines Hohlorgans z.B. des Darmes muß man die Volumänderung unter isotonischen Bedingungen annehmen, wobei die Kontraktion der Ringmuskulatur eine Hauptrolle spielt.

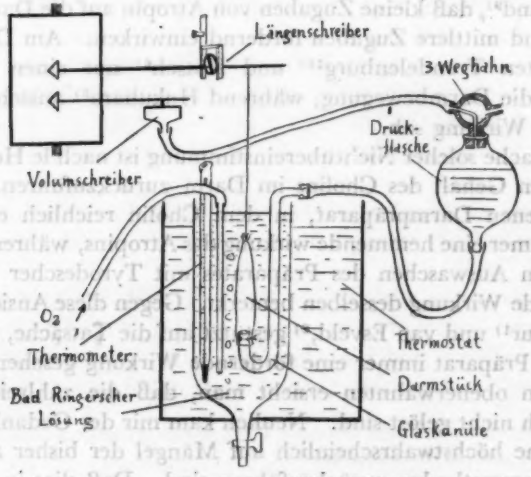

Abb. 1. Versuchsanordnung. Erklärung im Text.

Um die mich nun beschäftigende Frage zu lösen, wählte ich die Volumänderung unter isotonischen Bedingungen als geeigneter Indikator. Die Versuchsanordnung stimmt im Prinzip mit der Trendelenburgschen überin, wie es in Abb. 1 angegeben ist. Das untergebundene orale Ende dees

ausgeschnittenen Darmstückes wird mit dem Längenschreiber verbunden, während das anale Ende desselben mittelst der zweimal gekrümmten Glaskanüle und des Gummirohres mit der Druckflasche, die mit der Ringerschen Lösung* gefüllt ist, verbunden wird. Die obere Öffnung der Flasche ist mit Hilfe eines Dreiweghahnes und des Gummirohres mit der Mareyschen Registrierkapsel verbunden worden. Das Darmstück befindet sich in einem zylindrischen Gefäß, das mit Ringerscher Lösung von 100 cm.³ gefüllt ist. Die Temperatur der Lösung ist durch den Thermostat auf 39°C ghalten. Als Ernährungsflüssigkeit bedient man sich manchmal auch der Tyrodeschen Lösung, was aber auf das Versuchs resultat keinen Einfluß ausübt. Der Innendruck des Darmes ist in den einzelnen Versuchen zwischen 2 bis 3 Wasserhöhe gehalten. Als Prüfungsmittel bedient man sich eines Atropin-sulfats (Merk).

Ausserdem wurden einige Versuche über den Dünndarm der verschiedenen Tiere in situ ausgeführt, wobei zur Registrierung der Darmbewegung sowohl die Trendelenburgsche[14] als auch die Straub und Viaudsche Methode[22] angewandt wurde.

Versuchsergebnisse.

Kaninchen.

A. Der ausgeschnittene Dünndarm. Die Versuche wurden an 30 Darmstücken ausgeführt, die von 11 Tieren stammten. Bei kleinen und mittleren Dosen von Atropin bemerkte ich immer eine fördernde Wirkung. Die kleinste Dosis, die gerade die Darmerregung hervorrufen kann, ist 0.025 mg. in 100 cm.³ Badeflüßigkeit, während die größte Dosis, die noch

Abb. 2. Einfluß des Atropins auf den ausgeshnittenen Kaninchendünndarm. Versuch: 19, Juli, 1947. L_1 Langenänderung. V_1 Volumänderung. L_2 Längenänderung eines anderen Darmstückes, das nach der Magnusschen Methode in demselben Gefäss suspendiert worden ist. Der Pfeil zeigt den Moment der Zusetzung der Atropinlösung. Die Zeitmarkierung in 6 Sekunden (Erklärung im Text).

erregende Wirkung auf den Darm ausüben kann. ungefähr 30 mg. in 100 cm.^{3} ist. Wenn man darüber hinaus die Dosis von Atropin steigert, so geht die Bewegung des Darmes in Lähmung über. Die erregende Wirkung von Atropin auf den Darm veranschaulicht Abb. 2 und besonders die Volumenkurve V_1.

Ohne Atropin zeigt sich eine periodisch wiederkehrende Vergrößerung und Verkleinerung der Kontraktionswellen. Bei einer Dosis von 15 mg. Atropin treten an vereinzelten Stellen des Darmes, obwohl leichten Grades, tonische Kontraktionen auf, wodurch der Verlauf der normalen Kontraktionswellen beeinträchtigt wird. Dementsprechend steigt das Niveau der Volumenkurve, während die Amplitude der Volumänderung kleiner wird. Im Laufe von 6–7 Minuten lösen sich diese tonischen Kontraktionen allmählich. Die Kontraktionswellen werden dabei nach und nach stärker, bis sich schließlich fast alle Kontraktionswellen gleichstark und regelmässig von oral nach anal fortpflanzen.

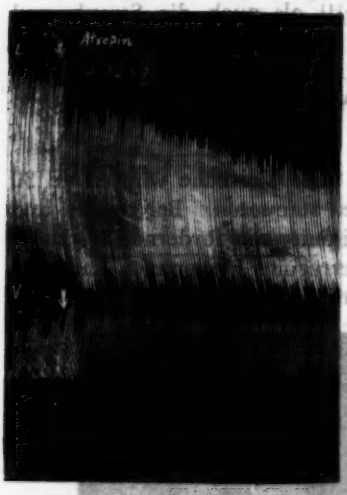

Abb. 3. Die Regularisierung der Darmbewegung durch Atropin. Versuch: 17, Juli, 1947. L Längenänderung. V Volumänderung. Nach Gaben von Atropin werden die Volumschwankungen größer und gleichmässiger. Zeitmarkierung in 6 Sekunden.

Wie in Abb. 2 und an der Längenkurve L_1 dargestellt, kann man ein Herabsinken vom Niveau der Längenkurve bemerken, das mit der Verkleinerung der Amplitude der einzelnen Bewegungen begleitet ist und diese Verkleinerung wird um so deutlicher, je größer die Amplitude der Volumänderung wird. An einem anderen neben dem obenerwähnten gestellten Darmstück, zeigt sich nach der Magnusschen Methode eine ähnliche Längenänderung, wie an dem obenerwähnten, was in Abb. 2 L_2 zu sehen ist. Wenn man hierbei die Volumänderung nicht berücksichtigt und nur, gestützt auf die Längenkurve L_1 oder L_2, die Bewegungsänderung des Darmes beurteilt, so hält man es für höchst wahrscheinlich, daß das Atropin auf die Darmbewegung einen hemmenden Einfluß ausübt. Und gerade das war die irrtümliche Auffassung der bisherigen Autoren.

Wenn man nun weiter 25 mg. Atropin zusetzt, so verkleinert sich die Volumänderung, ohne eine merkliche Längenänderung hervorzurufen. Man bemerkt nämlich eine Hemmung in der Bewegung. Bei der Magnusschen Methode sieht man eine Vergößerung der Amplitude der Bewegung,

wenn auch die peristaltischen Wellen in Wirklichkeit abgeschwächt werden was Abb. 2 L_3 veranschaulicht.

In Abb. 3 sieht man bei Zugabe von Atropin fast gleiche Bewegungsänderung wie bei dem obenerwähnten Fall, d. h. die Verstärkung und die Regularisierung der Bewegung. Am Anfang dieses Versuches bemerkt man am oralen sowie am analen Ende des Darmes zwei Pulsationen, woraus sich zwei Konraktionswellen in entgegengesetzter Richtung an der Darmwand fortpflanzen, um sich in der Mitte des Darmstückes zu vereinigen und in eine leichte Kontraktur zu geraten. Bei Zugabe von Atropin verstärken sich allmählich die am oralen Ende auftretenden Wellen, um schließlich die am analen Ende anftretenden Wellen vollständig zu unterdrücken. Dazu verschwindet auch die Irregularität der Amplitude der einzelnen Kontraktionen. Eine solche Regularisierung der Bewegung ist eine charakteristische Wirkung des Atropins. Die zu dieser Kategorie gehörende Bewegungserscheinung wurde in 12 Fällen beobachtet.

Dagegen kann man, wenn es auch verhältnismäßig selten der Fall ist, durch kleine Dosen Atropin die Extrakontraktionen längere Zeit dauernd an den einzelnen Stellen des Darmstückes auftreten sehen, wobei die rechtläufigen und rückläufigen peristaltischen Wellen zusammenstoßen und somit wird die Regelmäßigkeit der Bewegung stark beeinflußt. Außerdem kann man manchmal bemerken, daß die Periode der Gruppenperistaltik und die Periode der Pause oder diejenige der geschwächten Bewegung abwechselnd auftreten, wie es bei dem ausgeschnittenen Meerschweinchendarm gewöhnlich der Fall ist, Ich beobachtete dieses in 4 Fällen.

Manchmal kann man bei Atropinzugabe auch die deutliche Tonuszunahme und Verstärkung der einzelnen Bewegungen in der Längsrichtung auftreten sehen, was Abb. 4 veranschaulicht.

Gestützt auf die obenerwähnten Resultate kann man in bezug auf die fördernde Wirkung des Atropins auf den ausgeschnittenen Kaninchendünndarm zusamenfaßend aussagen:

1) Die kleine und mittlere Dosis von Atropin wirkt sowohl auf die Längs-als auch die Ringmuskelschicht des Dünndarmes fördernd, und sogar in der Regel stärker auf die letztere.

2) Die mittlere Dosis von Atropin verkleinert die vorhandenen Tonusschwankungen, macht einzelne Erregungswellen stärker und unterdrückt die Extrakontraktionen wenn sie vorhanden sind. Das Atropin entfaltet nämlich eine Wirkung, die man die Regularisierung der Bewegung nennen kann.

B. Der Kaninchendünndarm in situ. Nach der Methode von Straub und Viaud wurden 3 Versuche gemacht, wobei sich die Beweglichkeit des

Dünndarmes so herabsetzt, daß sich nur eine schwache Volumänderung erkennen läßt. Nach kleinen und mittleren Atropinzugaben tritt niemals eine Hemmung der Bewegung auf, sondern nur eine Förderung derselben.

Nach der Trendelenburgschen Methode wurden 4 Versuche gemacht. Bei einer kleinen Dosis von 0.1 mg. bis zu einer großen Dosis von 70 mg. wurde der Tonus des Darmes stets gesteigert und auch die einzelnen rhythmischen Bewegungen gefördert.

Die obenerwähnten Resultate stimmen mit den der Trendelenburgschen Versuchen[19] nicht überein. Die Ursache des Widerspruches ist aber bis jetzt unaufgeklärt.

Meerschweinchen.

5 Versuche wurden ausschließlich an dem ausgeschnittenen Darm gemacht, wobei man eine deutliche erregende Wirkung des Atropins bemerkt, dessen kleinste erregende Dosis 0.01 mg. ist. Derausgeschnittene Meerschweinchendarm macht in der Regel unter dem Innendruck von 1 cm. Wasserhöhe keine Bewegung. Wenn man in diesem Zustand Atropin von 25 mg. auf den Darm wirken läßt, so kann man plötzlich eine

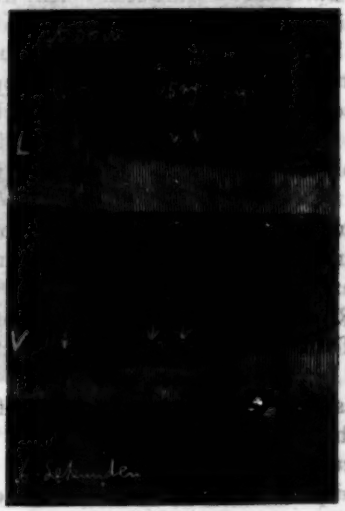

Abb. 4.

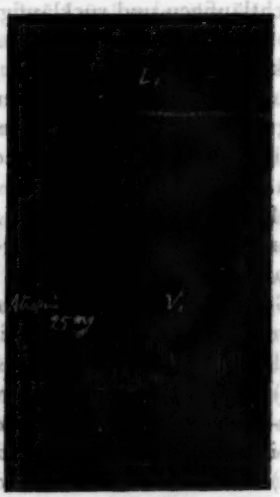

Abb. 5.

Abb. 4. Einfluß des Atropins auf den ausgeschnittenen Kaninchendünndarm. Versuch: 4, Nov., 1947. Bei diesem Versuch ist nicht nur die Amplitude der Volumänderung sondern auch diejenige der Längenänderung vergrößert worden.

Abb. 5. Einfluß des Atropins auf den ausgeschnittenen Meerschweinchendünndarm. Versuch: 11, Aug., 1947. Erklärung im Text.

augenfällige, gesteigerte Volumschwankungen bemerken, die durch die typische peristaltischen Wellen hervorgerufen werden, während interessanterweise die Längenänderung kaum bemerkbar ist (Abb. 5). Das ist darauf zurückzuführen, daß das Atropin beim Meerschweinchen wie beim Kaninchen hauptsächlich auf die Ringmuskulatur des Darmes erregend wirkt. Die obenerwähnten Resultate stimmen mit den der Trendelenburgschen nicht überein. Der Grund des Widerspruches ist noch nicht aufgeklärt.

Katze und Hund.

Das Verhalten des ausgeschnittenen Katzen-wie Hundedünndarms ist in dem Bad mit Ringerscher oder Tyrodescher Lösung durchaus nicht normal. Man kann zwar bei einem Innendruck von 3.5 cm. Wasserhöhe in der Regel nur noch schwache rhythmische Längenänderungen auftreten sehen, aber kaum eine Volumänderung. Kleine und mittlere Dosen von Atropin wirken auch bei diesen Tieren in 6 Fällen wie beim Kaninchen und Meerschweinchen immer erregend. Die kleinste erregende Dosis war in einem Fall 0.025 mg., aber gewöhnlich wirkt es erst bei mehr als 1 mg. sicher fördernd. Man sieht nach kleinen Atropingaben stets eine deutliche Steigerung des Tonus nach der Längsrichtung und auch die darauf superponierten Längenänderung, während erst nach mittleren Atropingaben (20–30 mg.) die Ringmuskulatur auch in Erregung gesetzt wird, um die Volumschwankungen größer zu machen.

Aus den diesen Tatsachen kann man folgern, daß das Atropin auf den Dünndarm der Katze wie des Hundes erregend wirkt und sogar auf die Längsmuskulatur stärker. Ob die Empfindlichkeit der Ringmuskulatur auf das Atropin in dem völlig physiologischen Zustand des Darmes doch noch unter derjenigen der Längsmuskulatur steht, ist aber sehr fraglich.

Zusammenfassung.

Der Einfluß des Atropins sowohl auf den ausgeschnittenen als auch den noch sich im Körper befindenden Dünndarm der verschiedenen Säugetiere, des Kaninchens, des Meerschweinchens, der Katze und des Hundes wurde mit Hilfe der verschiedenen Methoden (Trendelenburg und Straub und Viaud) untersucht: die Resultate konnte folgendermaßen zusammengefaßt werden.

1) Was die Änderung der Länge des Darmrohres anbelangt, so arbeiten die beiden Darmmuskelschichten unter isotonischen Bedingungen antagonistisch: Wenn sich die Ringmuskulatur stärker kontrahiert, so verlängert sich die Länge des Darmes wahrscheinlich mit der verkleinerten Amplitude der Längenbewegung. Diese muß aber nur als eine scheinbare Hemmung betrachtet werden, weil die Darmkraft dabei in Wirklichkeit

durch die Kontraktion der Ringmuskulatur gesteigert worden ist. Damit man also die Beförderung oder die Hemmung der Darmbewegung durch irgendein Arzneimittel beurteilen kann, muß man jedenfalls nicht nur die Längenänderung des Darmrohres, sondern auch die Volumänderung untersuchen. Bei den Untersuchungen über die Atropinwirkung auf den ausgeschnittenen Dünndarm haben die meisten Autoren allerdings die Magnussche Methode angewandt, mittelst deren man aber nur die Längenänderung des Darmstückes registrieren kann. Daß die Widersprüche unter den Autoren in der methodischen Unvollkommenheit zu suchen ist, kann man wohl mit Recht sagen.

2) Nach kleinen und mittleren Dosis von Atropin (0.1–25 mg. in 100 cm.³ Badeflüßigkeit) wird der ausgeschnittene Dünndarm bei den verschiedenen Säugetieren stets fördernd beeinflußt. Beim Kaninchen und Meerschweinchen wirkt das Atropin auf die Ringmuskulatur stärker als die Längsmuskulatur erregend. Bei der Katze und dem Hund aber wirkt das Atropin auch auf die Längsmuskulatur stark erregend.

3) Die kleine und mittlere Dosis von Atropin (0.1–70 mg. intravenös) übt auf den Kaninchendünndarm in situ stets fördernden Einfluß aus.

Diese Untersuchung wurde auf Kosten der Ausgaben des Unterrichtsministerium für naturwissenschaftliche Forschung ausgeführt, wofür ich an dieser Stelle meinen besten Dank aussprechen möchte.

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* Die Ringersche Lösung wurde nach Hukuhara's Angabe (Jap. J. Med. Sci., part 3, Biophysics, 1943, **8**, 235) hergestellt und hat die folgende Zusammensetzung: NaCl 0.85, $CaCl_2$ 0.025, KCl 0.021, $NaHCO_3$ 0.020, Aq. dest. 100.00.

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Über die Automatie des Sino-Atrial-Ringes des Warmblüterherzens.

Von

Takesi Hukuhara und **Syomatu Yokoyama.**

(福原 武) (横山 正松)

(Aus dem Physiologischen Laboratorium der Medizinischen Fakultät zu Yonago.)

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Aus dem Gedanken heraus, daß am Säugetierherzen die ringsum die sinoaurikulare Grenzfurche liegende Muskulatur der Sitz der Automatie sein könnte, entdeckten Keith und Flack[6] den ihnen benannten Knoten. Daß dieser Knoten der Sitz der höchsten automatischen Fähigkeit des Herzens ist, ist zwar eine wohl begründete Tatsache, man kann jedoch nach dem gegenwärtigen Stand der Forschung in bezug auf die Automatie anderer Stellen des obenerwähnten Sino-Atrial-Ringes (Si.-A.-Ring) nichts bestimmtes sagen. Einige physiologische Versuche machen es wahrscheinlich, daß die bestimmten Stellen des erwähnten Ringes doch noch mit automatischer Fähigkeit begabt sind: Wir[4] haben neuerdings eine mit Automatie begabte Stelle an der dem Ansatz der der völlig zurückgebildeten Eustachischen Klappe entsprechenden Stelle des Hundeherzens gefunden. Nach Schlomovitz und Chase[12] wird die Herzbeschleunigung durch die Erwärmung der rechten und vorderen Stelle der Einmündung der unteren Hohlvene des Hundeherzens hervorgerufen.

Anatomisch heben einige Autoren hervor, daß eine spezifische Muskulatur nicht nur an der Sinusknotengegend, sondern auch an den anderen Stellen des Si.-A.-Ringes vorhanden ist: Nach Pace[7], Segre[9] und Bruni[1] umgreift der Sinusknoten beim Schaf, Mensch, Wiederkäuer und Pferd die obere Hohlvenenmündung hufeisenförmig; ein Schenkel geht aus dem Kopfteil und zieht in die Gegend des Vorhofsseptums. Takahashi[10] sagt, daß eine spezifische Muskulatur den unteren Rand der unteren Hohlvenenmündung des menschlichen Herzens umgreift.

Aus dem obenerwähnten physiologischen und anatmischen Untersuchungen ist ersichtlich, daß eine genauere Untersuchung notwendig wird, um die automatischen Fähigkeit und die Ausdehnung des Si.-A.-Ringes klarzustellen. Dies veranlasste uns die folgenden Untersuchungen auszuführen.

Das Herz verschiedener Warmblüter, des Kaninchens, des Hundes

und der Katze wurde ausgeschnitten und mit dem nicht mit Ringerscher Lösung verdünnten defibrinierten Hundeblut durchströmt. Die Bewegung des rechten Herzohres und der Herzspitze wurde mit Hilfe der Suspensionsmethode auf der berußten Trommel registriert. die Erwärmung der Ansatzstellen der Sinusklappen geschieht mittelst einer Thermode, durch die auf 55°C erwärmtes Wasser strömt. Der Durchmesser der Thermodenspitze beträgt dabei 2 mm. Genauer sind die obenerwähnten Methodiken schon in den vorigen Mitteilungen (2), (3), u. (4) beschrieben worden.

Die Ausschaltung des Sinusknotens* wurde folgendermaßen erreicht: Abb. 1 veranschaulicht dies: Am oberen Rande der Einmündung der unteren Hohlvene macht man einen Schnitt in die Vorhofswand senkrecht zum Sulcus terminalis, wobei der Schnitt darauf weiter abwärts bis zum unteren Drittel der Vorhofswand verlängert wird (a). Von diesem Schnitt anfangend werden weitere Schnitte beiderseits vom Sulcus terminalis, demselben parallel und zwar 2–3 mm. entfernt nach vorne hin bis zum Vena Cava-Herzohr-Winkel geführt (b und c), und dann ein Schnitt, der zum Sulcus terminalis senkrecht steht und dem Vorhofsseptum sehr nahe liegt (d), gemacht. Der so gebildete viereckige Lappen enthält eine allgemein anerkannte Sinusknotengegend.

Damit man die Ansatzstellen der Sinusklappen erwärmen kann, wird ein weiterer Schnitt an der oberen Wand der unteren Hohlvene gemacht (e). Wenn man dann die rechte Wand der Vene und die hintere Vorhofswand nach rechts, so lassen sich alle Stellen der Sinusklappen erkennen,.

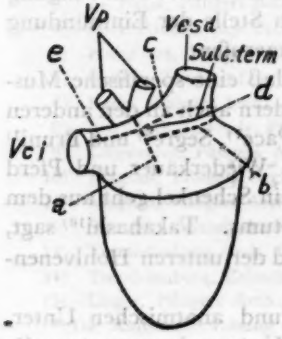

Abb. 1. Schema des Warmblüterherzens, die die Art des Schneidens der rechten Vorhofswand bei der Ausschaltung der Sinusknotengegend zeigt. Vcsd V. cava sup. dext. Vci V. cava inf. Vp V. pulmonalis. Erklärung im Text.

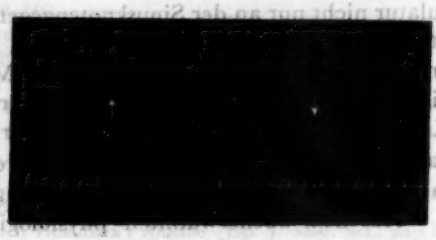

Abb. 2. Einfluß der Erwärmung der Ansatzstelle der Eustachischen Klappe auf die Schlagfrequenz des Kaninchenherzens.

Versuchsergebnisse.

I. Physiologisches.

A. Kaninchenherz.

Wenn man am Kaninchenherzen den Sinusknoten ausschaltet, so tritt eine Verlangsamung der Herzschläge ein. Dabei ist die Schlagperiode 1.02 bis 1.90 fach größer als die normale.

Bei dem des Sinusknotens beraubten Herzen erwärmt man die Ansatzstellen der einzelnen Sinusklappen. Die Resultate sind folgendermaßen zusammengefaßt.

1) Die Erwärmung der Ansatzstelle der Eustachischen Klappe (14 Fälle) ruft immer eine merkliche Beschleunigung der Herzschläge hervor, wobei die Herzfrequenz in den meisten Fällen nicht nur diejenige vor der Erwärmung, sondern auch die normale weit übertreffen kann. Abb. 2, zeigt ein Beispiel.

2) Bei der Erwärmung der Ansatzstelle der Thebesiusschen Klappe (11 Fälle) bemerkt man immer eine merkliche Beschleunigung der Herzschläge. Ausgenommen einiger Ausnahmefällen ist der Grad der Beschleunigung gleich dem bei der Erwärmung der Eustachischen Klappe.

3) Durch die Erwärmung des unteren Abschnittes der linken Sinusklappe (10 Fälle) kann eine Beschleunigung der Herzschläge zwar manchmal sehr merklich erfolgen, aber sie ist häufig nicht so bedeutend wie bei der Erwärmung der obenerwähnten Klappen.

4) Bei der Erwärmung des mittleren Abschnittes der linken Sinusklappe (3 Fälle) kann man noch eine geringe Beschleunigung der Herzschläge bemerken.

5) Die Erwärmung des oberen Abschnittes der linken Sinusklappe ruft nur selten eine vorübergehende Beschleunigung einiger Pulse hervor, aber kein überzeugender Effekt macht sich dabei bemerkbar.

Nach den obenerwähnten Resultaten kann man mit Recht sagen, daß der Si.-A.-Ring beim Kaninchenherzen, den oberen Abschnitt der linken Sinusklappe ausgenommen, der Sitz der Automatie ist und sogar der Ansatz der rechten Sinusklappe und derselbe des unteren Abschnittes der linken vor allem mit bedeutender automatischer Fähigkeit begabt sind.

B. Katzenherz.

Wenn man am Katzenherzen den Sinusknoten ausschaltet, so tritt immer eine Herzverlangsamung geringeren Grades ein. Darauf erwärmt man die Ansatzstellen der einzelnen Sinusklappen. Durch die Erwärmung sowohl der Eustachischen als auch der Thebesiusschen Klappe kann immer

eine merkliche Beschleunigung der Herzschläge hervorgerufen werden. Was die automatische Fähigkeit der linken Sinusklappe anbelangt, so kann man an ihrem unteren Abschnitt zwar sehr häufig einen mehr oder weniger merklichen Herzbeschleunigungseffekt bemerken, aber an ihren oberen und mittleren Abschnitten kaum.

Die Ausdehnung der Zentren, die im Si.-A.-Ring ihren Sitz haben, ist also beim Katzenherzen kleiner als beim Kaninchenherzen; es fehlt nämlich die Automatie der linken Sinusklappe nicht nur an ihrem oberen Abschnitt, sondern auch an ihren mittleren Abschnitt.

C. Hundeherz.

Am Hundeherzen, dessen Sinusknoten vorher ausgeschaltet worden ist, ruft die Erwärmung der Gegend der schon völlig rückgebildeten Eustachischen Klappe manchmal eine merkliche Beschleunigung der Herzschläge hervor und die Erwärmung der Gegend der in den meisten Fällen ebenfalls völlig rückgebildeten Thebesiusschen Klappe ruft kaum einen Beschleunigungseffekt hervor, was schon in der vorigen Abhandlung mitgeteilt worden ist.

Bei der Erwärmung der anderen Stellen des Si.-A.-Ringes konnte man in allen mit Vorsicht untersuchten Fällen (15 Fälle) gar keinen Einfluß auf die Herzschläge bemerken.

II. Histologisches.

Nach den obenerwähnten physiologischen Untersuchungen liegt es nahe zu vermuten, daß man an der Stelle des Si.-A.-Ringes die mit der Automatie begabt ist, eine spezifische Muskulatur finden kann, was in der Tat der Fall war. Bei den histologischen Untersuchungen wurde folgendermaßen verfahren: Am ausgeschnittenen Herzen des Versuchstieres wurden die einzelnen Schnitte, die in Abb. 1 gezeigt sind, gemacht, um den Si.-A.-Ring bloßzulegen. Darauf wurde das Herz in der Ciaccioschen Lösung fixiert, in Paraffin gebettet und daraus wurden Serienschnitte von 10μ Dicke in frontaler Richtung gemacht. Jeder zehnte Schnitt wurde erst mit Hämatoxylin dann nach van Gieson gefärbt.

Der Si.-A.-Ring zweier Kaninchen wurde histologisch untersucht, wobei übereinstimmende Resultate erzielt wurden. Am Si.-A.-Ring des Kaninchenherzens kann eine spezifische Muskulatur nicht nur im Sinusknoten, sondern auch am Ansatz sowohl der Eustachischen als auch der Thebesiusschen Klappe gefunden werden. der Schwanzteil des Sinusknotens endet nicht am oberen und vorderen Einmündungsrand der V. cava inferior, sondern er verläuft weiter unten dem rechten Einmündungsrand derselben

entlang, in anderen Worten der Ansatzstelle der rückgebildeten Eustachischen Klappe entlang. Am hinteren Rand des Sinus coronarius geht die Eustachische Klappe in die Thebesiussche Klappe, die den unteren Rand der Öffnung der V. cava sup. sin. umrandet, über. Am Ansatz dieser Klappe kann man ebenfalls eine Gruppe spezifischer Muskelfasern bemerken.

Am Ansatz der linken Sinusklappe, die am hinteren Rand der Fossa ovalis ihren Sitz hat, konnten wir zwar eine Gruppe kleiner Muskelfasern bemerken, aber wir konnten nicht ihren Spezifität mit Sicherheit nachweisen. Am oberen Abschnitt der linken Sinusklappe kann eine solche Muskelgruppe nicht mehr wahrgenommen. Ausserdem ist eine Verbindung der spezifischen Muskulatur im Si.-A.-Ring mit dem Tawaraschen Knoten nicht bemerkbar.

Die Ausdehnung und die Verteilung spezifischer Muskulatur des Si.-A.-Rings ist beim Katzenherzen ungefähr ebenso wie beim Kaninchenherzen. Es ist dabei bemerkenswert, daß sich die spezifische Muskulatur am Ansatz der rechten als auch die an der linken Sinusklappe am vorderen Rand der Öffnung des Sinus coronarius ohne Unterbrechung miteinander verbinden.

Zwei Hundeherzen wurden histologisch untersucht. Der Schwanzteil des Sinusknotens endet am oberen und vorderen Einmündungsrand der unteren Hohlvene und sein weiterer Ausläufer konnte an dem rechten Einmündungsrand des genannten Gefäßes nicht mehr verfolgt werden. Daraus geht aber nicht hervor, daß keine spezifische Muskulatur an der physiologisch mit Automatie begabten Stelle des Si.-A.-Ringes, nämlich am Ansatz der rückgebildeten Eustachischen Klappe ihren Sitz hat, weil die Zahl der Versuche hierbei zu klein ist, um ein abschließendes Urteil zu gewinnen.

Besprechungen der Ergebnisse.

Aus den Resultaten der obenerwähnten physiologischen und histologischen Untersuchungen geht hervor, daß sich der Ort der Reizbildung beim Warmblüterherzen nicht nur an der Sinusknotengegend lokalisiert, sondern ein solcher sich auch über andere Stellen des Ringes verbreitet, und daß sich die Verhältnisse der Verbreitung je nach der Entwicklungshöhe des Tieres regelmäßig verändern. In Abb. 3 ist die Verbreitung der automatischen Zentren an dem Si.-A.-Ring verschiedener Tiere schematisch dargestellt: Beim Kaninchen, des sich unter den untersuchten Warmblütern auf der niedrigsten Stufe der Entwicklung befindet, ist der Si.-A.-Ring, den oberen Abschnitt der linken Sinusklappe ausgenommen, noch mit einer merklichen automatischen Fähigkeit begabt. Bei der

Katze, die höherentwickelt ist als das Kaninchen, fehlt es im oberen und mittleren Abschnitte der linken Sinusklappe an Automatie. Bei dem noch höher entwickelten Hunde bleibt die automatische Fähigkeit nur noch an der Eustachischen Klappe übrig, während sie an der linken Sinusklappe völlig verloren gegangen ist.

Abb. 3 A. Schematische Darstellung der automatischen Zentren an dem Si.-A.-Ring des Kaninchenherzens.
Die mit Automatie begabten Stellen sind punktiert.

Aus den obenerwähnten Tatsachen kann man vermuten, daß der Si.-A.-Ring bei einem Warmblüter, der sich auf einer noch niedrigeren Stufe der Entwicklung als das Kaninchen befindet, reingsum der Sitz der Automatie sein könnte.

Was die Automatie des Si.-A.-Ringes des menschlichen Herzens anbelangt, so liegt es nahe zu vermuten, daß der Ort der Reizbildung an dem Si.-A.-Ring nicht weiter als beim Hunde verbreitet sein konnte. Mit dieser Vermutung aber stehen die Resultate der histologischen Untersuchungen von Takahashi[10] teilweise in Widerspruch, weil er an der Stelle des Si.-A.-Ringes, dem unteren abschnitte der linken Sinusklappe, wo es beim Hunde an Automatie völlig fehlt, einen aus spezifischer Muskulatur bestehenden Knoten gefunden hat. Wir fügen bei dieser Gelegenheit noch hinzu, daß Wakayama[11] am menschlichen Herzen die Befunde von Takahashi nicht bestätigen konnte.

Zusammenfassung.

Um die automatische Fähigkeit der einzelnen Stellen des Sino-Atrial-Ringes der Warmblüterherzen festzustellen, führten wir am Kaninchen-, Katzen- und Hundeherzen physiologische sowie histologische Untersuchungen aus. Bei einer Serie von Versuchen werden die Stellen des Si.-A.-Ringes, durch deren thermische Reizung ein Herzbeschleunigungseffekt hervorgerufen werden kann, in anderen Worten die mit Automatie begabten Stellen, festgestellt. Bei einer anderen Serie von Versuchen wurde

der Si.-A.-Ring ringsum in Serien geschnitten, damit man feststellen kann, ob eine spezifische Muskulatur an den mit Automatie begabten Stellen vorhanden ist oder nicht. Die Resultate werden folgendermaßen zusammengefaßt:

1) Beim Warmblüterherzen geht die automatische Fähigkeit des Si.-A.-Ringes gemäß der Entwicklungsstufe des Tieres, und zwar in folgender Reihenfolge, verloren: Zuerst fehlt dem oberen Abschnitt der linken Sinusklappe die Automatie (Kaninchen), dann dem mittleren Abschnitt derselben (Katze), und weiter dem unteren Abschnitt sowie dem Ansatz der Thebesiusschen Klappe (Hund). Beim letztgenannten Tier bleibt der Ansatz der schon völlig rückgebildeten Eustachischen Klappe noch als der Sitz der Automatie übrig.

2) Bei allen untersuchten Warmblüterherzen ist der Sinusknoten der Ort der höchsten Automatie. Der Anastz sowohl der Eustachischen als auch der der Thebesiusschen Klappe haben beim Kaninchen und Katzenherzen eine gleich große automatische Fähigkeit, und dann kommt die Fähigkeit der linken Sinusklappe.

3) An den Stellen, die physiologisch mit Automatie begabt sind, kann man im allgemeinen eine spezifischer Muskulatur finden: Beim Kaninchen-und Katzenherzen kann man am Ansatz der Eustachischen und Thebesiusschen Klappen mit Sicherheit eine spezifische Muskulatur feststellen, während man an dem der linken Sinusklappe mit Wahrscheinlichkeit das Vorhandensein einer solchen annehmen kann. Beim Hundeherzen konnten wir aber am Ansatz der Eustachischen Klappe eine spezifische Muskulatur nicht bemerken.

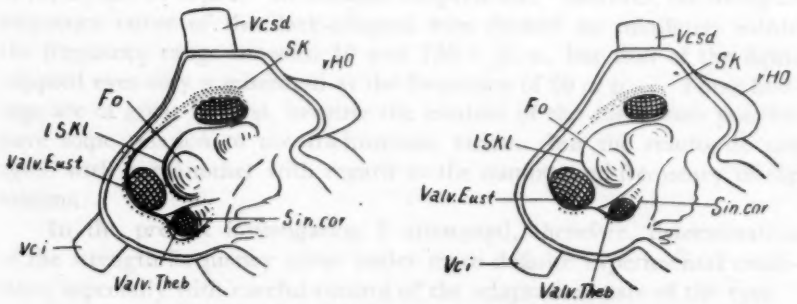

Abb. 3 B und C. links Katzenherz, rechts Hundeherz.

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* Bei der Beschreibung der Lage der Herzteile denken wir, daß die Basis der Kammern horizontal, die Spitze des Herzens abwärts gerichtet ist und daß Herzseptumm sagittal gestellt ist.

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Influence of Adaptation on the Strength-Frequency Curve of Human Eyes, as Determined with Electrically Produced Flickering Phosphenes.

By

Zensuke Abe.
(阿部 善助)

(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai.)

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Introduction.

A sinusoidal alternating current of low frequency passing across the human head causes a subjective light sensation of flickering phosphenes. Barnett[1] determined the thresholds for first appearance of the flicker by gradually increasing the intensity of the current and found that the strength-frequency relation consisted of three intersecting V-shaped curves with minima at 22, 7 and 4.5 cycles per sec. (c. p. s.).

Motokawa and Iwama[2] reported that the strength-frequency curve obtained by stimulation with alternating currents and repetitive rectangular pulses of a direct current has three minima at the frequencies of 18, 36 and 54 c. p. s. In Schwarz's experiment,[3] however, the strength-frequency curve of the dark-adapted eyes showed no minimum within the frequency range between 10 and 120 c. p. s., but that of the light-adapted eyes only a minimum at the frequency of 20 c. p. s. These findings are of great interest, because the minima of the curve may possibly have some relation to the trichromatic vision. But the results do not agree with one another with regard to the number and frequency of the minima.

In the present investigation I attempted, therefore, determination of the strength-frequency curve under more definite experimental conditions, especially with careful control of the adaptation state of the eyes.

Experimental.

Method.

The arrangement used for electrical stimulation is represented schematically in Fig. 1. Two silver electrodes, 2.5×1.5 cm.^{2} in size, were

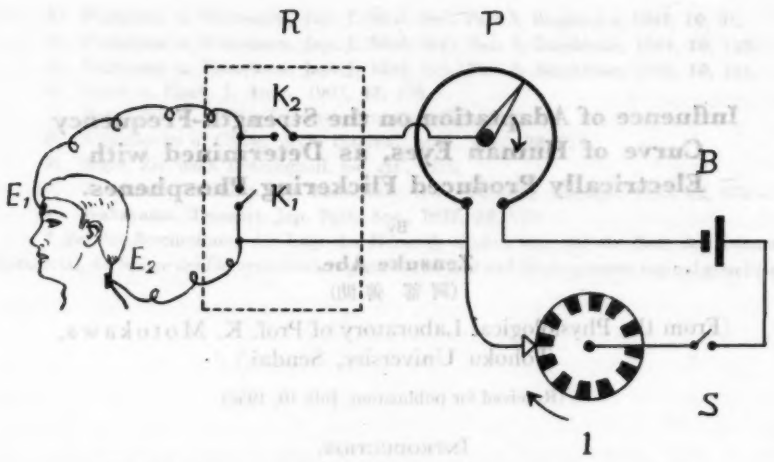

Fig. 1. Arrangement for measurement. E_1, E_2: Electrodes. R: Rheotome. K_1, K_2: Keys. P: Potential divider. I: Interrupter. B: Battery. S: Switch.

placed at the forehead and the occiput and fixed by a bandage, the electric contact between skin and electrodes being secured by adhesive paste containing much NaCl. Stimulating currents were generated by revolution of an interrupter (I), so that they consisted of rectangular pulses following one after another with the same interval as the duration of a current pulse.

Their frequency ranged between 1 and 120 c.p.s. and was controlled accurately by means of a stroboscope. Two different methods were employed for determination of the threshold. In the 1st method, the threshold voltage for the first appearance of the flicker was taken, when the voltage was gradually increased at the rate of 0.08 volts per sec. In this case, of course, the rheotome (R) in Fig. 1 was not used. In the second method, keeping the duration of the stimulating current at a constant value of 0.5 sec., the threshold value was determined by the method of trial and error. The rheotome (R) was used for this purpose. Data obtained by these two methods were qualitatively almost the same, but some systematic differences were found, especially in the range of high frequencies, as will be shown later. The discrepancy may be related to such an adaptation-like phenomenon of the flickering phosphenes at higher frequencies, as reported by Pollock and Meyer.[4]

The illumination of the whole visual field for the purpose of light adaptation was made as uniform as possible, and sometimes a small circular patch, subtending 4.7° at the eye, was used for illumination of a

circumscribed retinal area in darkness. Two colored filters, one transmitting lights of wave-lengths longer than 710 mμ and the other those of wave-lengths shorter than 490 mμ, were used for illumination with red and blue-violet lights respectively.

Results.

I. Experiments on light-adapted eyes.

Schwarz[8] showed that the optimum condition for observation of flickering phosphenes is a weakely illuminated state of the retina or an early stage of dark adaptation following a strong pre-illumination of the eye.

In order to make measurements under such optimum conditions the following experiment was carried out at the moment 1 minute 30 seconds after the end of a pre-illumination of 24,000 lux with the 1st method. The data taken in this way are plotted as ordinates against frequencies as abscissæ in Fig. 2 (curve L). The curve L has five minima at 7, 20, 36, 55 and 75 c.p.s. The most distinct minimum at 20 c.p.s. will correspond to the minimum at 22 c.p.s. found by Burnett and to that at 18 c.p.s. re-

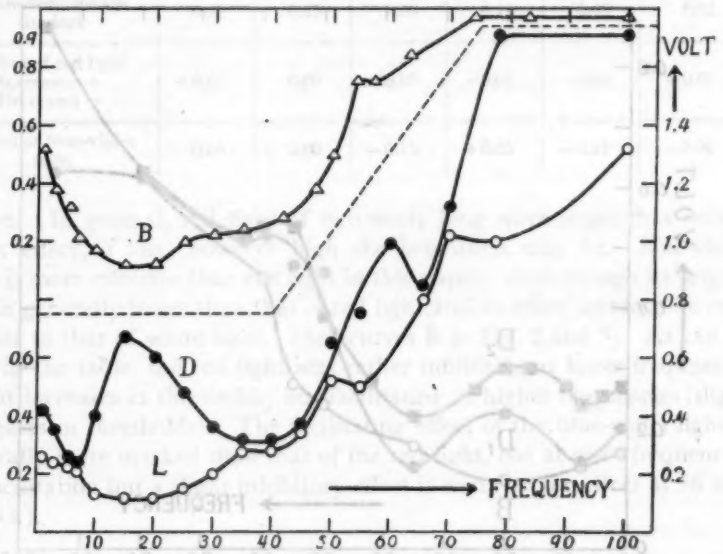

Fig. 2. Results by first method. Ordinate: Threshold value in volts, Absciassa: Cycles per sec. Curves L and D denote experiments on light-and dark-adapted eye respectively. Curve B effects of illumination of a small retinal area.

ported by Motokawa and Iwama. The minimum at 7 c.p.s. is found least distinct.

II. Experiments on dark-adapted eyes.

After preliminary dark adaptation of 30 minutes the strength-frequency curve was determined. The result obtained with the 1st method is represented by the curve D in Fig. 2. As can be seen from this figure, the form of the curve is quite different from that of the curve L; two distinct minima at 7 and 36 c.p.s. came in sight and a new minimum at 65 c.p.s. appeared instead of the two minima at 20 and 54 c.p.s. found in the curve L. This characteristic of the extremely dark-adapted eyes was confirmed further by the 2nd method as shown in Fig. 3 (curve D_1 and D_2). In general, the electric threshold of the dark-adapted eye is higher than that of the moderately light-adapted eyes over the entire range of frequencies, and the magnitude of the difference in thresholds at any frequency varies with frequencies in such a manner that the maximum differences are seen around 1, 15 and about 55 c.p.s. and the minimum differences at 7, 35 and 65 c.p.s.

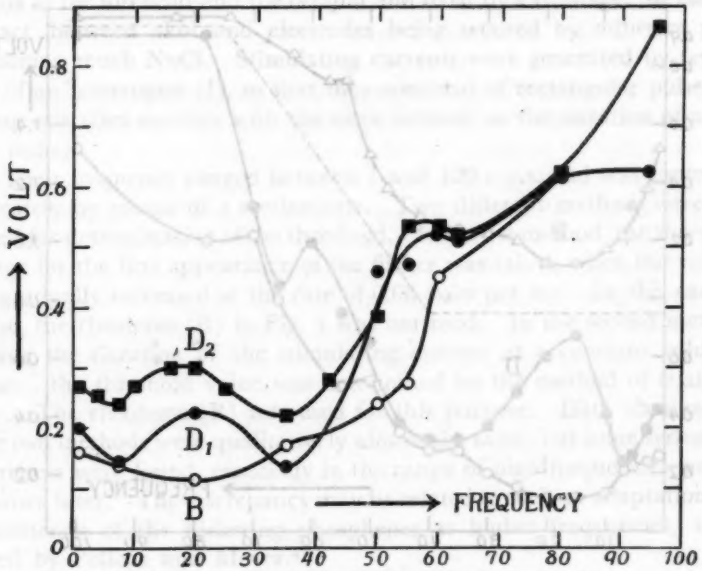

Fig. 3. Results by second method. Curves D_1 [Subject: T. Mita] and D_2 (Subject: Z. Abe) denote experiments on dark-adapted eye. Curve B effects of illumination of a small retinal area.

III. Effects of illumination of a small retinal area.

After sufficient dark adaptation, measurements of the electric threshold were made under continued exposure of the central part of the retina to the small illuminating patch stated above. When the brightness of the white patch was very low, the strength-frequency curve was not at all influenced by illumination. But when its brightness was sufficiently raised, a curve corresponding to the curve L in form was obtained, showing that a localized illumination was sufficient to bring the retina into such a state as yielded a strength-frequency curve of light-adapted form.

When a small patch of red or blue-violet light was used instead of white, the effect on the form of the curve was found different according to colors. In Table I the examples obtained by the 2nd method are

TABLE I.

Effects of Illumination with Colored Lights upon Electric Thresholds at Varying Frequencies.

Frequency of stimulating currents	1	7	20	36	50	79
Threshold voltage in dark	0.21	0.15	0.26	0.13	0.45	0.62
Effect of red light Increase: + Decrease: −	+0.02	0.00	+0.03	−0.02	−0.04	−0.01
Effect of blue-violet light	−0.04	0.00	−0.15	+0.05	−0.12	−0.01

shown. In general, red light of extremely long wave-length has only a slight effect, if any, however high the brightness may be. Blue-violet light is more effective than red light in this respect, even though its brightness is generally lower than that of red light and its effect seems to be very similar to that of white light. (See curves B in Fig. 2 and 3) As can be seen in the table, the red light acts rather inhibiting at lower frequencies (slight increases in thresholds) but facilitating at higher frequencies (slight decreases in thresholds). The facilitating effect of the blue-violet light is generally more marked than that of the red light, but at some frequencies no facilitation but a slight inhibitory effect is seen (see the effect at 36 and 7 c.p.s.).

DISCUSSION.

Motokawa and Iwama[2] emphasized the harmonic relation between three optimum frequencies of 18, 36 and 54 c.p.s. found in the curve of the

light-adapted eyes. And they proposed a resonance hypothesis, assuming three kinds of resonators in the eyes. In the present experiment it has, however, been found that such a harmonic relation holds to a certain extent for the data obtained from the light-adapted eyes, but scarcely for the data obtained from the extremely dark-adapted eyes. The fact that the two minima at 7 and 35 c.p.s. depend little on adaptation, while the minimum at 20 c.p.s. disappears under complete dark adaptation, is worth of attention in theoretical consideration of this phenomenon. If resonators are to be assumed at all, five or more resonators should be assumed in the human retina. In view of the findings stated above, some of the resonators must be modified in their function by the effect of light falling on the retina, but others not.

According to Barnett,[1] 7 c.p.s. should be the optimum frequency for electrical stimulation of those portion of the optic tracts connected to rods and 22 cycles the corresponding optimum frequency for those portions connected to the cones. This inference seems to be based on the fact that the minimum at 22 c.p.s. appears in bright light, whereas the minimum at 7 c.p.s. appears in dim light. But such a conclusion is not acceptable for the following reasons.

If the fact that the minimum at 20 c.p.s. can be seen under illumination by bright white light suggested a close relation between this minimum and cone-function, it should be expected that the same minimum manifests itself also under illumination by red light strong enough to stimulate cones. In reality, however, no such minimum could be observed under such conditions of illumination in my experiment stated above.

As shown above, electric thresholds for dark- and light-adapted eyes show the greatest difference at about 20 c.p.s., that is, the effect of light upon the electrical threshold is most marked at this frequency. The same relation can be observed also in the fact that the electric threshold for the current of 20 c.p.s. changes in the course of dark adaptation following a strong pre-illumination to far greater extent than to thresholds for currents of any other frequencies. For example, the time course of changes in the electrical threshold for the current of 20 c.p.s. after pre-illumination of 24000 lux was such that at first the threshold remained low and steady for about 5–7 minutes, and then increased rapidly to attain a maximum in about 17 minutes. A similar time course of electrical excitability was obtained also by Iwama[5] using a constant current pulse of about 50 msec. in duration for stimulation. Comparing the time courses of dark adaptation in electrical excitability and in photosensitivity of the retina with each other we see that the rapid increase in the electric threshold sets in at about the same moment as the abrupt decrease in the light threshold begins. Since this change in photosensitivity is obviously associated with

rod-funktion the rapid increase in electrical excitability during dark adaptation should be connected with rod-function as well.

The absence of the minimum at 20 c.p.s. must be due to the low electrical excitability of rods in the completely dark-adapted state, and its presence in earlier stages of dark adaptation or in moderate light adaptation can be ascribed to the electrical supernormal excitability of retinal elements acquired by moderate light adaptation of photoreceptors. The supernormal excitability is the very property which Motokawa[6] utilizes in his analysis of color-reception. The fact that blue-violet light is effective in lowering the electrical threshold while red light of extremely long wave-lengths has little effects, will find its explanation in the circumstance that blue-violet light can stimulate rods, whereas red light in scarcely effective in this sense.

For the role played by rods for production of the minimum at 20 c.p.s., more conclusive evidence has been established by Tukahara and Abe and will be reported in the following paper.

SUMMARY.

The relation between the threshold intensity, as determined with the light-sensation evoked by electrical stimulation of the eyes, and the frequency of the stimulating currents was studied in varying adaptation states of the eyes. The strength-frequency curve of the light-adapted eyes has 5 minima at frequencies of 7, 20, 35, 54 and 74 c.p.s. and that of the dark-adapted eyes has 3 minima at frequencies of 7, 35 and 65 c.p.s. The two kinds of strength-frequency curves taken under light- and dark-adaptation differ from each other most greatly at 20 c.p.s., moderately about 55 c.p.s., and only slightly at 7 and 35 c.p.s. Even though a small circumscribed area of the retina is illuminated, the strength-frequency curve varies its form in various manners according to the brightness and wave-lengths of light used. In general, red light of extremely long wave-lengths has only a slight effect on the form of the curve, but blue-violet light is far more effective. It was discussed that 20 c.p.s. may be the optimum frequency for electrical stimulation of those portions of the optic organs connected to rods.

Author wish to thank Prof. K. Motokawa and Assist. Prof. T. Mita for their good advice and cordial guidance.

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On the Essential Factors of Motokawa's Method for Measuring Fatigue.

By

Tosisada Mita, Zensuke Abe and **Tzoe Byonshik.**
(三田俊定)　(阿部善助)　(崔秉植)

(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai.)

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Introduction.

Motokawa and Suzuki reported a new method for measuring fatigue, in which the main procedure is as follows: Repetitive rectangular pulses of electric current of 20 cycles per sec. are applied for stimulation of the subject's eye in such a manner that the voltage is increased at a constant rate from zero to such a level as the subject perceives distinct flickering phosphenes, and then decreased at the same rate until he feels no flicker. Readings of the stimulating voltages are taken at the moments, when the subject gives signals informing the appearance and the disappearance of the flicker, in order to obtain both sorts of threshold for appearance (S_1) and for disappearance (S_2). Motokawa and Suzuki proposed to use the difference (ΔS) between these two threshold values, i.e. S_1-S_2 as a measure of fatigue.

As shown by Motokawa[1] and his collaborators[2,3] this measure ΔS seems to some extent useful as an indicator of fatigue, but no research on its physiological significance has ever been carried out. In the present investigation effects of illumination, frequency of current pulses and rate of raising and lowering the stimulating voltage on the measure ΔS were investigated to make clear the dependence of the measure on various physiological factors.

In view of a weak point of the original method that the value of ΔS depends on such an unessential factor as the reaction time of the experimenter, it was attempted to exclude this factor from ΔS in this exeprriment.

Experimental.

Method.

Fig. 1 shows the arrangement used for measurements schematically.

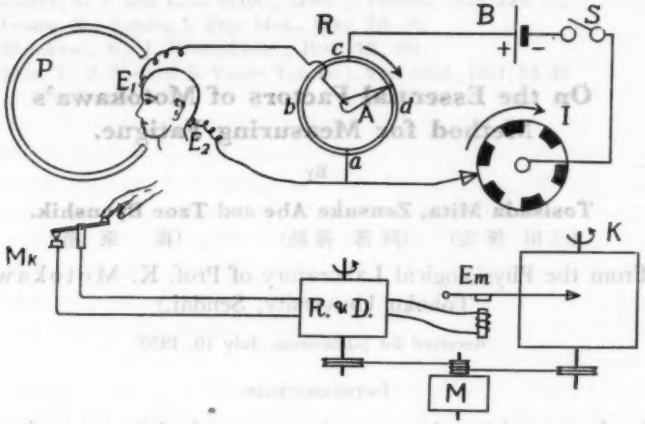

Fig. 1. Arrangement for measurement. A: Revolving contact arm of the rheonome; B: battery of 2 volts; D: apparatus devised for recording the revolutions of rheonome; Em: electromagnet; E_1, E_2: electrodes for stimulation; I: interrupter for generating the repetitive rectangular pulses of stimulating current; K: kymograph; M: electric motor; Mk: Morse's key; P: projector for illumination; R: rheonome; S: switch.

The stimulating voltage was raised and lowered automatically by means a rheonome (R) instead of manual operation in the original method. When the pointed contact arm (A) of the rheonome was revolved at a constant speed along its circular path which served as a fluid resistance (150 ohms). The voltage between two electrodes (E_1 and E_2) was increased and decreased alternately at a constant rate in such a manner that when the contact arm moved from point *a* through *b* to *c* on the circular path of the rheonome, the stimulating voltage increased from zero to maximum, and decreased from maximum to zero by moving along the other half path (*c*—*d*—*a*).

The subject used a Morse's key (Mk) for sending the signals as soon as he perceived appearance and disappearance of the flicker. The signals were recorded on a kymograph (K) by means of an electromagnet (Em), as shown in Fig. 1. The rate of revolution of the rheonome itself was also recorded at the same time by one and the same electromagnet (Em) in such a way that the current of the electromagnet was interrupted at definite intervals. By virtue of this recording system the stimulating voltage at any moment could be found on the kymogram, and thus both sorts of threshold voltage (S_1 and S_2) could be determined without involving the reaction time of the experimenter.

The interrupter (I) for production of constant current pulses was

similar to that used by Motokawa et al. The rheonome, kymograph and interrupter were driven by a electric motor through a pulley system. The rate of revolution was accurately controlled by means of a stroboscope. Experiments were carried out in a dark room under artificial illumination whose intensity ranged from 1 to 10^3 lux. However some measurements were made in open air to study effects of high intensities of illumination. The illumination in the whole visual field was kept as uniform as possible. Measurements were taken after the subject's eyes had become adapted to the illumination. Each of the three authors served in turn as a subject. All these experiments were performed under good physiological conditions of subjects so as to avoid implications of fatigue and other difficult factors.

Results.

I) Effects of frequencies of current pulses.

Effects of frequencies on both sorts of threshold value (S_1 and S_2) and on their difference (ΔS) were studied at various levels of adaptation of the eyes. Two examples of these measurements are represented in Table I,

TABLE I.

Effects of Frequencies of Current Pulses at Various Levels of Adaptation of the Eyes.

Experimental conditions	Frequency in c.p.s.	10	14.3	20	25	33.3
illum: 10^3 luxes	S_1 in volt	1.37	1.09	0.90	0.99	1.39
V : 0.1 volt/sec.	S_2 "	1.06	0.83	0.70	0.78	1.10
subj. Abe	ΔS "	0.31	0.26	0.20	0.21	0.29
illum: 1 lux	S_1 "	1.13	1.05	1.02	1.25	1.66
V : 1 volt/sec.	S_2 "	0.92	0.89	0.90	1.01	1.19
subj. Mita	ΔS "	0.21	0.61	0.12	0.24	0.47

which shows that each value of S_1, S_2 and ΔS is minimum at the frequency of 20 cycles per sec., and that the larger the values of S_1 and S_2, the larger becomes ΔS. This finding indicates that a constant frequency is a necessary condition not only for the determination of S_1 and S_2 but also for that of ΔS.

II) Adaptation state.

The effects of adaptation state on the values of S_1, S_2 and ΔS were investigated. As the eyes are adapted to illuminations of varying intensities, values of S_1, S_2 and ΔS vary roughly parallel to one another, and

TABLE II.

Effects of Adaptation State.

$S=(S_1+S_2)/2$, V: 0.067 volts per sec. Subject: Abe.

	Dark	10 luxes	7000 luxes
ΔS	0.38 volts	0.14 volts	0.34 volts
S	1.09 „	0.39 „	0.89 „
$\Delta S/S$	0.35	0.35	0.38

the measure ΔS is found approximately proportional to the arithmetic mean (S) of S_1 and S_2 at each level of adaptation. An example of these measurements is illustrated in Table II. As can be seen in this table both values of S and ΔS are minimum under the illumination of 10 lux, and the ratio $\Delta S:S$ remains approximately constant inspite of marked changes in ΔS and S with variations of illumination.

This relation was further confirmed by measuring ΔS and S at each stage of dark adaptation following a strong pre-illumination. These findings indicate that measurements of ΔS for testing fatigue should be made under a constant illumination. However, the measure ΔS depends relatively little on illumination at such low intensities as 1–100 luxes, when the rate of raising and lowering of stimulating voltage is properly chosen, for example at a rate 80 millivolts per sec. Under such experimental conditions, changes in ΔS due to changes in illumination remain within 30%.

III) Rate, V, of raising and lowering the stimulating voltage.

The relation of both sorts of threshold value (S_1 and S_2) to the rate (V) of raising and lowering the stimulating voltage is represented graphically in Fig. 2 A, which shows that S_1 and S_2 have a linear relation to V respectively at each level of adaptation. Hence the difference ΔS has also a linear relation to V, as shown in Fig. 2 B. This relation is expressed by the following experimental formula,

$$\Delta S=S_1-S_2=E+2TV \quad (1),$$

where E and T are constant at a given level of adaptation. It deserves attention that threshold values for disappearance S_2 are negative at higher values of V, as can be seen in Fig. 2 A. The reason for this curious effect will be mentioned later.

IV) Reaction time, T, for the minimum stimulus.

The value of the proportionality coefficient T of the formula (1) can be obtained graphically from the slope of the lines in Fig. 2 B. The data so obtained are represented in Table III. T has the dimension of time

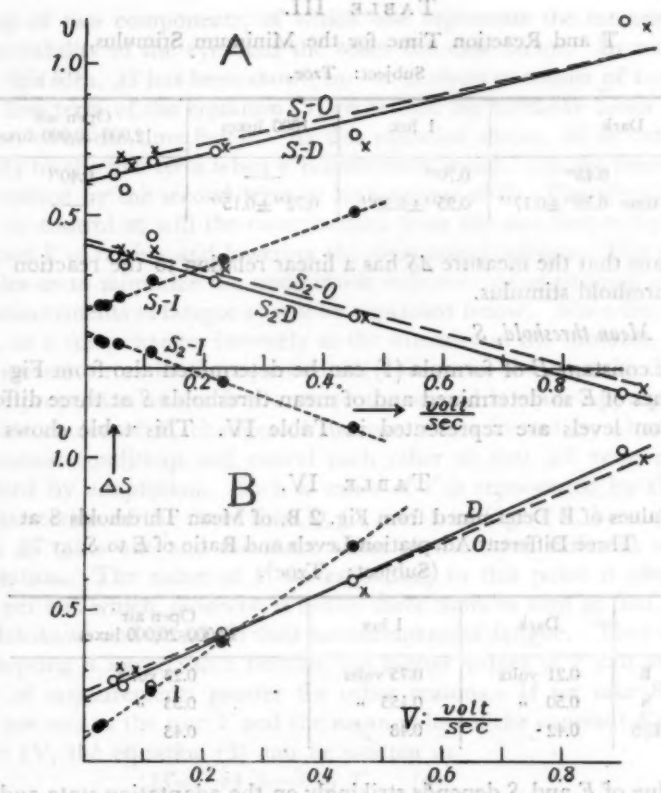

Fig. 2. A Ordinate: threshold value in volts. Abscissa: rate (V) of raising and lowering the stimulating voltage in volts per sec. S_1 and S_2: the threshold values for appearance and for disappearance of the flickering phosphenes respectively. O, D and I denote three adaptation levels, i.e. in open air (crosses), in complete darkness (empty circles) and under illumination of 1 lux (solid circles) respectively.

B Linear relations between ΔS and V in three adaptation states. Notations D, O and I as in A.

and its value is of the same order as the value of reaction time obtained for very weak stimuli. The values of reaction time for the minimum stimulus obtained from one and the same subject under experimental conditions similar to those in the above experiment are shown in the same table for comparison with the values of T. It can be seen in this table that both sorts of data agree well with each other at all adaptation levels of the eyes.

TABLE III.

T and Reaction Time for the Minimum Stimulus.

Subject: Tzoe.

	Dark	1 lux	1000 luxes	Open air 12,000–20,000 luxes
T	0.45″	0.70″	——	0.40″
reaction time	0.59″±0.17″	0.95″±0.30″	0.72″±0.15″	——

This means that the measure ΔS has a linear relation to the reaction time or the threshold stimulus.

V) Mean threshold, S.

The constant E of formula (1) can be determined also from Fig. 2 B. The values of E so determined and of mean thresholds S at three different adaptation levels are represented in Table IV. This table shows that

TABLE IV.

Values of E Determined from Fig. 2 B, of Mean Thresholds S at Three Different Adaptation Levels and Ratio of E to S. (Subject: Tzoe).

	Dark	1 lux	Open air 12,000–20,000 luxes
E	0.21 volts	0.75 volts	0.23 volts
S	0.50 "	0.155 "	0.53 "
E/S	0.42	0.48	0.43

each value of E and S depends strikingly on the adaptation state and that the ratio of the two kinds of values is constant irrespective of adaptation. This can be expressed by the equation.

$$E=KS, \quad (2),$$

here K is a constant independent of adaptation. Substituting E in (1) by (2), we get

$$\Delta S=KS+2TV \quad (3)$$

This formula shows that ΔS depends not only on the reaction time T but also on the mean threshold, S. This equation implies the parallelism between ΔS and S which has been shown above experimentally under varying conditions of adaptation as well as at varying frequencies of stimulating currents.

DISCUSSION.

Motokawa and Suzuki discussed that ΔS must be a measure con-

sisting of two components, of which one represents the intensity factor of excitability of the eyes and the other the time factor. In accordance with this idea, ΔS has been shown by our analysis to consist of two terms. The first term of the equation (3) represents the intensity factor and the second term the time factor. As this equation shows, ΔS is determined mainly by the first term when V is sufficiently small. On the contrary it is determined by the second term at high values of V. Therefore it is possible to control at will the contributions from the two factors by varying the rate V of raising and lowering the stimulating voltage. This situation enables us to minimize the undesirable influence of adaptation upon ΔS in measurements of fatigue as will be explained below. Since the reaction time, as a rule, changes inversely as the intensity of the stimulus, the first and second terms in the equation (3) change in the opposite direction. If the proportion of the first term to the second is made appropriate by suitable choice of V, changes of the two terms caused by alteration of adaptation conditions will cancel each other so that ΔS remains uninfluenced by adaptation. Such a value of V is represented by the point of intersection of the three lines D, O, and I in Fig. 2 B, because at this point ΔS takes one and the same value in the three different states of adaptation. The value of V corresponding to this point is about 0.25 volts per sec. which, however, is nearly three times as high as that adopted by Motokawa and Suzuki in their measurements of fatigue. They are right in adopting a lower value because too higher values of V can make the error of measurements greater for other reasons. If we take 80 millivolts per sec. as the rate V and the mean value of the constant K given in Table IV, the equation (3) can be written as

$$\Delta S=0.44\ S+0.16\ T \qquad (4)$$

where S and T are to be given in volts and in sec. respectively.

In cases of complete dark and extreme light adaptation S is about 0.5 volts and T is about 0.45 sec. as shown in Table III and IV. We know from these data and equation (4) that under these conditions of adaptation the mean threshold is the determining factor for ΔS. In case of moderate light adaptation such as under illumination of several lux the term including the reaction time is predominant.

In measurements of fatigue which are usually carried out indoors both factors must contribute equally to the measure ΔS. This argument furnishes us the theoretical ground for the usefulness of the method by Motokawa and Suzuki, because it enables us to get quantitative information about both intensity- and time factors of excitability, as suggested by Motokawa.

It must be emphasized that the reaction time involved in these measurements is the one for threshold stimuli. We can easily understand that

such a particular reaction time is much more sensitive to fatigue than such reaction times for stronger stimuli as have been taken as a measure of fatigue. The method is very practical because one can determined the two measures, i.e. threshold and reaction time by a single procedure and express them by a single index ΔS.

It must, however, be borne in mind that both threshold and reaction time depend upon a number of factors known and unknown other than fatigue. For instance, the reaction time of normal subjects generally increases with falls of body temperature, as reported by Kleitman.[4] Such a temporary decrease in ΔS after dinner as reported by Motokawa and Suzuki may partly be due to a rise in body temperature.

SUMMARY.

Motokawa's method for measuring fatigue takes as an index of fatigue a measure ΔS, which represents the difference between the threshold for appearance (S_1) and that for disappearance (S_2) of the flicker evoked by respective electrical stimulation of the eye. In the present investigation it was found that ΔS can be analyzed into two factors, the mean threshold (S) and the reaction time (T) for the minimum stimulus, where S is the arithmetical mean of S_1 and S_2. The relation can be expressed by the following formula,

$$\Delta S = KS + 2TV,$$

where K is constant and V the rate of raising and lowering the stimulating voltage. As indicated by this relation, two important factors S and T are embodied in a single measure ΔS which can be measured very easily by the Motokawa's method. Thus, our analysis has provided a theoretical ground for the usefulness of this method, and at the same time, a clue to the choice of experimental conditions such as illumination, the rate of raising and lowering the stimulating voltage, etc.

We wish to thank Prof. K. Motokawa for his good and cordial advice.

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Biochemical Studies on Carbohydrates.

CXXXIX. On Non-amino Sugars in Serum Proteins.

By

Humi Inazawa.

(稲津 ふみ)

(From the Medico-chemical Institute, Tohoku University, Sendai.
Director: Prof. H. Masamune.)

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In several papers since 1928, Bierry[1] claimed to have isolated mannose (as phenylhydrazone) as well as galactose (as methylphenylhydrazone) from the hydrolysates of the carbohydrates separated from plasma proteins of different animals. However, the descriptions as to the procedures of isolation and data for identification were too short for accurate judgement. For the presence of mannose in those carbohydrates, Dische's[2] (1928) and Rimington's[3] (1929) report are accepted as conclusive evidences, but as to the presence of galactose only indirect proofs have been afforded as by Sörensen and Haugaard[4] (orcinol method on serum-albumin and -globulin by means of step photometer), Rimington[5] (orcinol method on serum-mucoid by means of Hilger Spekker absorptiometer), Suzuki[6] (simple colorimetry by diphenylamine, indole and orcinol methods on the carbohydrate from crude serum-mucoid), Werner and Odin[7] (paper partition chromatography of "serum-mucoid") and Masamune and Yosizawa[8] (paper partition chromatography of serum-mucoid).

The investigations above indicated that the carbohydrates of various serum proteins including the mucoid are mutually identical and contain the two non-amino sugars in an equimolecular proportion besides glucosamine. Hence the writer prepared, to separate the sugar components, crude serum mucoid which possesses a comparatively large amount of the carbohydrate. The material was subjected to acid hydrolysis and the non-amino sugar fraction free from glucosamine was obtained by the aid of mercuric acetate and baryta. Therefrom mannose was precipitated with phenylhydrazine and, in addition, galactose with methylphenylhydrazine and the sugars were identified after purification of the precipitates. The yields of the both products nearly matched each other in number of molecules.

Experimental.

Crude Serum-mucoid as a Material.

It was prepared according to Rimington[5] with minor modifications:— Bull serum, diluted with an equal volume of 0.9% NaCl and adjusted to pH 4.7, was heated for 2 hours in a boiling water-bath and stood for 1 day (As a preservative 2 cc. of alcoholic 20% thymol solution were added per liter of the dilute serum) before filtration. The clear filtrate was distilled in vacuo to less than half the volume and dialyzed for 3 days against running water (Thymol again added). Some precipitating material was filtered off and the filtrate further subjected to vacuum distillation to a syrupy consistency, eliminating half way a few precipitates that occurred hereby. To the condensate were added 5 volumes of abs. alcohol and 2 volumes of ether, and the deposits were dried in a desiccator. After many runs 8 g. of substance were given in total. The yield was 0.2 g. per *l.* of the blood. The mixture contained 11.5% N, 5.0% non-amino sugar, as expressed as comprising equal parts of mannose and galactose, and 15.6% ash.

Isolation of Mannose and Galactose.

Two attempts were effected. The second one which ran more beautifully will be described below.

Preliminary hydrolysis and fractionation.

Satoh and others'[9] procedure in their study of vitellin carbohydrate was applied:—

3.21 g. of the dry material were heated with 100 cc. of 1 N H_2SO_4 for 5 hours (under reflux) on a boiling water-bath. To the hydrolysate, when cold, were added at first 15 g. of crystalline baryta and then the saturated baryta to neutral. The precipitates were centrifuged off and the supernatant liquid (about 250 cc.) distilled in vacuo to about 130 cc. The precipitates appearing during the distillation were again removed by filtration and washed with water. Then, to the filtrate and washings (150 cc. in total) 26 cc. of 25% mercuric acetate were added and after standing for several hours, the mixture was neutralized carefully with the saturated baryta, whereupon yellow precipitates came out. Stood overnight and centrifuged. The supernatant liquid and the washings of the centrifugate were united, barium was cast down with 5 N H_2SO_4, added drop by drop till 3.4 of pH, and subsequently the centrifuged supernatant liquid was freed from mercury by the aid of H_2S gas[10] and condensed to about 20 cc. by vacuum distillation (the pH was lowered to about 2.5). It was further liberated from acetic acid by passing ether in a continuous extractor (pH was elevated to 5.6)[11] and divided into two

equal parts, one for isolating mannose and the other for isolating galactose.

Separation of mannose as phenylhydrazone.

One half of the non-amino sugar fraction above was distilled to about 2 cc. under diminished pressure, 70 mg. of sodium acetate (anhydrous) dissolved and 5 cc. of water containing 100 mg. of phenylhydrazine hydrochloride added in drops. Cloudiness occurred soon. The mixture was stood for 4 hours at room temperature (10°C) and overnight in an ice chest and the crystalline deposits brought onto a suction funnel, washed with water, abs. alcohol and ether in sequence. F. P. 194–197°C. This crude product (69 mg.) was taken up in a smallest possible quantity of boiling water and filtered hot. Crystallization began immediately in the filtrate. After one day the crystals were collected and recrystallized furtheron three times from hot 70% alcohol, when the substance obtained possessed a constant melting point of 200°C. Mixed melting point test exhibited no depression. Non-colored, glistening, rhombic, thick plates. Yield, 48 mg.

N (Friedrich).
4.118 mg. substance, 0.424 mg. N.
$C_{12}H_{18}N_2O_5$. Calc., 10.37%; found, 10.30%.
Optical rotation in pyridine.

$$[\alpha]_D^{13} = \frac{0.118 \times 3}{0.01279 \times 1} = +27.44° \text{ (no mutarotation).}$$

Separation of galactose as methylphenylhydrazone.

a) Conditions suitable for separating galactose as methylphenylhydrazone in the co-presence of mannose.

Methylphenylhydrazine gives the mono-hydrazone of mannose, which is difficultly soluble, though less difficultly than that of galactose. Therefore conditions needful to easily attain the purpose were looked for prior to the undertaking on the protein fraction.

Exp. 1. A mixture of each 50 mg. of mannose and galactose was dissolved in 3 cc. of water and 3 cc. abs. alcohol, 0.15 cc. methylphenylhydrazine and 0.15 cc. 50% acetic acid were added in the order named. The precipitates separated were washed with alcohol and ether and dried. They (125 mg.) melted at 175–178°C and involved two kinds of crystal, nearly rectangular plates and wide needles.

Exp. 2. 100 mg. of a similar sugar mixture were taken up in 3 cc. of water and after pipetting in 0.15 cc. of 50% acetic acid and 0.15 cc. of methylphenylhydrazine, drop by drop of abs. alcohol was added till homogeneous emulsion (no complete dissolution). The product amounted

only to 71 mg. Despite of its melting point of 175–178°C, not diverging from the above, almost all of the crystals had the needle form.

Exp. 3. 25 mg. each of mannose- and galactose-methylphenylhydrazone were stirred up with 7 cc. of water separately and stood at 40–45°C for 7–8 hours. The former hydrazone went into solution entirely, but the latter in part. So, in the next, a mixture of equal parts of the both hydrazones were dissolved in a smallest possible quantity of boiling water, followed by standing at 40–50°C. The derivative of mannose (plates) was retained in solution for several hours, whereas that of galactose came out quickly, and the precipitates, washed and dried, melted at 185–186°C.

From these results it seemed preferable for the aim in view to form galactose methylphenylhydrazone at first under restricting alcohol to a quantity as small as possible and to recrystallize the crude product from water by maintaining the solution between 40 and 50°C after boiling.

b) Separation from the protein-fraction.

The remaining half of the non-amino sugar fraction above (s. p. 54) was condensed by vacuum distillation to about 4 cc., and 0.15 cc. each of 50% acetic acid and methylphenylhydrazine were added with following addition of abs. alcohol sufficient to emulsify but insufficient to dissolve methylphenylhydrazine (About 1 cc. of abs. alcohol was required). The mixture was stood at 37°C for 6 hours (Crystallization began in 30 minutes) and then in an ice-chest overnight. Crystals all in needles and a small amount of amorphous substance deposited. They were collected together on a funnel with suction and washed with abs. alcohol and ether in sequence. 82 mg. was the crude product. F.P., 185–186°C. The amorphous contaminant (inorganic salts) which was insoluble in abs. alcohol was eliminated by boiling the product with this solvent so little as possible and filtering while hot (a hot water funnel). Again the filtrate was stood in an ice-chest overnight and the deposits separated. 71 mg. of crystals were given. The melting point was not elevated. The present product was purified three times by dissolving in boiling water (a smallest possible quantity) with immediate filtering and standing at 40°C. Here the melting point was raised but not above 187–188°C Further recrystallized from hot abs. alcohol, the substance melted a 191°C (the constant melting point). In the mixed melting point test happened no depression. Crop, 54 mg. Wide needles with silver luster truncated at both ends.

N (Friedrich).

4.112 mg. substance, 0.402 mg. N.

$C_{13}H_{20}O_5N_2$. Calc., 9.85%; found, 9.78%.

Optical rotation in pyridine.

32.64 mg. substance in pyridine filled up to 2 cc. showed no rotation at all (temp., 15°C).

SUMMARY.

From crude serum mucoid of bull were isolated mannose (as phenylhydrazone) and galactose (as methylphenylhydrazone) in nearly equimolecular amounts.

The costs of this work were defrayed by a grant from the Education Department (through the Grant Committee of the Science Council), which is gratefully acknowledged.

H. M.

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10) Precipitating mercuric sulfide was filtered on a funnel and washed with water. The washings were combined with the first filtrate.

11) Floating precipitates between the ethereal and watery layers were discarded.

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Rotatory Crossgraphy.

(Study on the Rotatography.)

Fourth Report.

By

Shinji Takahashi, M. Imaoka and **T. Shinozaki.**
(高橋 信次) (今岡 誼麿) (篠崎 達世)

(From the Department of Radiology, Hirosaki University School of Medicine, Hirosaki.)

(Received for publication, July 15, 1950)

We have reported four methods of knowing the transection of the living human body these four years.[1)2)3)] Now we are going to illustrate the new fifth one, Rotatory Crossgraphy, below.

Experimental.

Apparatus.

We designed and constructed two rotation tables A and B as shown in Fig. 1. The rotation axes of the two rotation tables and the focus of the X-ray tube are arranged so as to be in one vertical plane. The central X-ray, however, inclines fifteen degree to the surface of the rotation table B. The diameter of the rotation table A is 50 cm. and that of the rotation table B 30 cm. On the rotation table A a patient is seated, and on the rotation table B a film is placed. The height of the seat is regulated by the jack attached to the rotation table A. Both rotation tables rotate with the same angular velocity by an electric motor. In order to protect the patient from the X-ray injure and also to prevent the scattering of X-ray we place two lead slits and a Backy-Potters diaphragm at the front of the patient. The motor switch, X-ray switch and diaphragm starter are connected electrically and controlled from the controlboard.

Model Experiment.

1) We made experiments in order to know the optimum degree of the rotation for the purpose. We placed an aluminium tube eret on the rotation table A and operated the rotatory crossgraphy with the four following rotation angles (Fig. 2):

A) rotationangle of 20 degree; B) rotationangle of 60 degree;
C) rotationangle of 180 degree; D) rotationangle of 360 degree;

Fig. 1. Rotatory crossgraph in action.
T: X-ray tube.
D: Bucky-Potters diaphragm.
S: Lead slit.
TA: Rotation table A.
TB: Rotation table B.

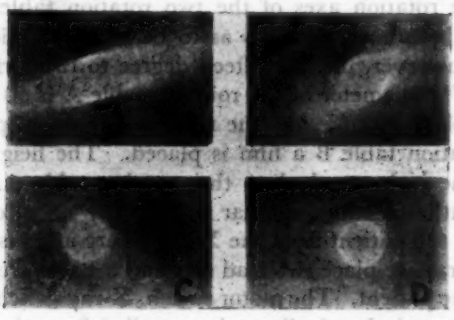

Fig. 2. X-ray images pictured under following rotationangles.
A) Rotationangle of 20 degrees.
B) 60 degrees. C) 180 degrees.
D) 360 degrees.

In the first case we got a crossgram with an X-ray image of a blade. In the second case an X-ray image of a spindle. Thus in the above two cases the figure of actual transsection was not got. In the third and fourth cases we got similar X-ray images of a circle, which are actual transections of the aluminium tube. From this experiment we learned

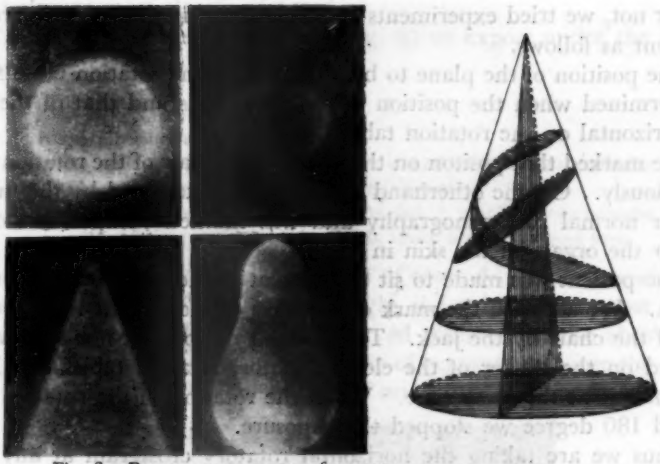

Fig. 3. Rotatory crossgrams of a cone.
A film being placed horizontally (A) inclined (B), erect (C) curved (D) Crossgrams of A, B, C and D (right) coincide to the conic sections (left) cut by the plane parallel to the film.

that the rotation table must be rotated at least 180 degree in the operation of the crossgraphy.

2) In order to know the relation between the position of the film and that of the photographed transection we tried next an experiment with a model. As the model we chose a cone of aluminium (Fit. 3). The cone was placed vertical on the rotation table A. The cone was 11.5 cm. high and the circle of the base 7.0 cm. in diameter. We placed a film on the rotation table B under four different conditions as follows.

A) With a cassette placed horizontally on the table.
B) With a cassette inclined 30 degree to the table surface.
C) With a cassette placed vertically to the surface.
D) With a specially made concave curved cassette packed with film.

We switched on the starter of the rotation table, and the tube current. When the rotation table rotated from 0 to 180 degree, we stopped the X-ray switch. Thus we got four leaves of rotatory crossgram (Fig. 3): which show the image of a circle (A), an ellipse (B), a hyperbola (C), and gourd shape (D). These figures coincide to the conic sections cut by the plane parallel to the film. Thus we learned that we have only to change the inclination or curvature of the film in order to get these X-ray images of the various section figures of the cone.

Human Body Experiment.

In order to know whether this method can be applied on the human

body or not, we tried experiments and got following results. The operation went as follows.

The position of the plane to be traversed on the rotation table A can be determined when the position of the tube focus and that of the film, laid horizontal on the rotation table B, are fixed.

We marked that positon on the back of the chair of the rotation table A previously. On the otherhand a patient was examined by the fluoroscopy or normal roentgenography and was marked the position of the focus or the organ on the skin in ink.

The patient was made to sit on the seat of the chair on the rotation table A. We adjusted the mark on the skin of the patient to that of the back of the chair by the jack. Then asking the patient not to move, we switched on the starter of the electric motor, rotation tables and X-ray tube current at the same time. When the rotation of the rotation table reached 180 degree we stopped the exposure.

Thus we are taking the horizontal rotatory crossgram at any parts of the adult human body. Technical conditions of our own experiences are as follows:

	Distance between Focus-Rotation-table A. cm	Rotation-table A–B. cm	KV	mAs	r-dosage irrad. to the skin	Magnifying rate
Head	82	40	80	450	9	1.48
Neck	82	40	70	200	2	1.48
Chest	146.5	48	80	360	3	1.33
Abdomen	65	40	80	450	14	1.61
Thigh	146.5	48	80	240	2	1.33
Leg	146.5	48	70	200	2	1.33

The X-ray image of the rotatory cossgram at several parts of the body is pretty good in gradation. The crossgram of the head distinctly revealed accessory nasal sinuses, zigomatic arc, orbit, pyramid and so on. When we take the crossgram at the height of the larynx, the transection of the spine and the complicated structure of the larynx are imaged on the film. In the case of the chest we got the clearly distinguished X-ray image of the A. pulm., trachea, heart, Aorta, sternum, ribs, scapula, spine. etc.

When we shift the position of the tubefocus higher, so as the central X-ray falls with inclination of 30 degree to the film, which is placed horizontal on the rotation table B, then we get the X-ray image in which ribs are shifted into the lung field and are superimposed there. For the reason the findings are mistakable to determine. On the corossgram of the abdomen the stomach, colon, vertebra and so on are imaged distinctly. In the case of lower extremities, however, the transection of the femur, tibia

and fibula are most clearly pictured.

To take the inclined transection (Fig. 4.) we expose under the following formula:

$$I = a.\mathrm{cosec}\ \theta.$$

I is the tubecurrent.

a is the optimum tubecurrent when the central X-ray is perpendicular to the film, namely the film inclines to the tablesurface 75 degree.

θ is the angle formed by the plane of the film A which is perpendicular to the central X-ray and that of the film B, which is to be exposed here.

When θ approaches 0 degree, the value of cosec θ increases rapidly and ultimately becomes infinite. Accordingly the tubecurrent must be increased rapidly too. In taking the curved transection, the above mentioned formula is applied to the technique of exposure, because the curved plane is considered as the integration of small planes. (Fig. 5)

Fig. 4. Rotatory crossgram of the chest.
The chest being traversed by a plane inclined 40 degree to the level as shown in diagram bottom right.

Clinical Experience.

In order to know whether the rotatory crossgraphy will bear practical use or not, we wish to mention following examples.

We are now taking the rotatory crossgram of cavern in the tuber-

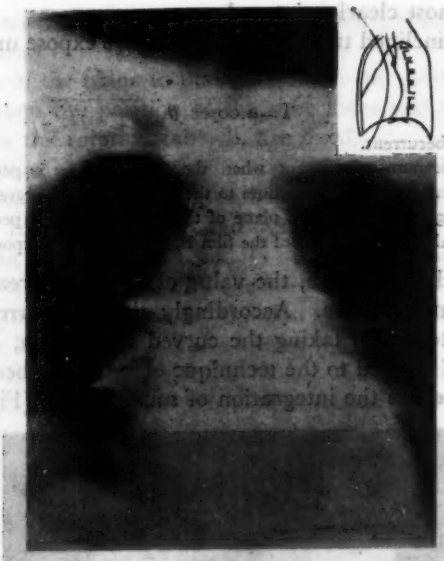

Fig. 5. Rotatory crossgram of the chest.
The chest being cut by a curved plane as shown in diagram top right.

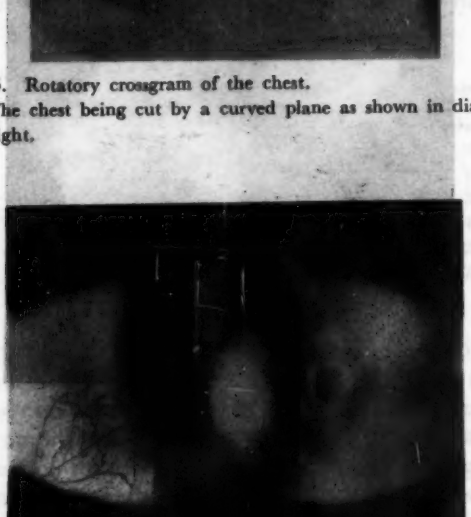

Fig. 6. Rotatory crossgram of the apex of the lung.
Normal roentgenogram reveals productive shadow in the upper part of the right lung and rounded shadow in the apex of the left lung. In the tran ection of the apex there is productive shadow in the central part of the right and a cavern in the vicinity of the ribs of the left lung.

culous lung as the routine work. (Fig. 6.) The rotatory crossgram reveals the cavern even when it is concealed by the infiltration and can not be found by the normal radiography.

The Lymphadenitis of the hilum, focus in the retrosternal place are found out only through this method and tomography. And at the same time the shape, the size or the position in the transection are found most cleary. The X-ray image of the cavern reveals its relation to the ribs. When the infiltration of the focus is extended just to the surface of the pleura, artificial pneumothrax cannot be undertaken with success.

Discussion.

Previously we reported four methods of taking radiograms of the transection in the body, namely Discontinuous and Continuous Rotatography,[1] Direct[2] and Indirect crossgraphy. These methods are characterized by the lead slit, equipped in the apparatus at the front of the patient and rotation of the rotation table from 0 degree to 180 degree in the operation. The body is traversed by the plane containing the tubefocus and the slit. The slit here serves as the cutter of the transsection. And the contour of the transection is drawn by the rotation of the body (or tubefocus) over 180 degree. The rotation here serves as the scraper of the contour. The rotatory crossgraphy newly introduced in this report differs from these methods in this respect that in place of the lead slit the optical slit, produced the geometrical law, acts as the cutter of the transsection.

On the other hand the rotatory crossgraphy differs from the tomography, stratigraphy in this respect that the X-ray tube is rotated around the patient over 180 degree and thus the contour of the transection is scraped completely. We published the report of study on the rotartoy crossgraphy on December 25th 1949 at the first meeting of Hirosaki Radiological Society.[3] Two months after the publication we received a reprinted paper of Dr. Gebauer from Prof. E. P. Pendergrass. Then we were informed for the first time that Dr. Gebauer[4] had reported his work last autumn on "horizontal Schichtaufnahme", which is similar to our study in principle. We worked, however, absolutely independent from him. Because of the occupation which naturally shut us completely from the works of foreign doctors. The difference between ours and his method goes as follows:

Difference of the technique	Dr. Gebauers	Our Method	Results.
1. Inclination of the central X-ray to the rotationtable	30°	15°	X-ray image without superimposed shadow

2.	Device to remove the scattered X-ray	none	Bucky-Potters Diaphragm and two slits	the at the front of patient	X-ray injure avoidable, good contrast in X-ray image.
3.	Rotationangle of rotationtable	360°	180°~190°		The effect of both methods is the same
4.	Inclination of the plane or curved film.	none	A device for the purpose is made.		Not only the horizontale transection, but also any transection of any inclination, even that of curved one.

The rotatory crossgraphy has clinical advantage over the customary roentgenography in making clear the state of the transection of the body which can never be informed by other methods. Because through the other old methods we can never tell whether the transection of a lead cylinder is a circle, an ellipse or a square.

SUMMARY.

A method, rotatory crossgraphy, which is capable of taking the radiogram of the horizontal or inclined, planed or curved transection of any part in the body, is described.

A tube focus and two rotation axes of a rotation table A for a patient, and a rotation table B for a film are arranged so as to be in one vertical plane. The central X-ray is made to incline 15 degree to the surface of the rotation table B. Rotating two rotation tables with the same angular velocity from 0 degree to 180 degree, we expose. In order to remove the scattered and harmful X-ray two slits and Bucky-Potters Diaphragm are equipped at the front of the patient. Model experiments and then human body experiments were tried and the optimum technique of the rotatory crossgraphy were studied.

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Studies on Melanin.

X. Incontinentia pigmenti (Bloch-Sulzberger).

By

Minor Ito.
(伊藤 實)

(From the Department of Dermatology, Faculty of Medicine, Tohoku University. Director: Prof. M. Ito.)

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In 1925 Bloch[1] reported a case of "hitherto undescribed congenital pigmentary disturbance" in a young girl under the title of "incontinentia pigmenti". Sulzberger[2] made in Bloch's clinic an extensive description on this subject in 1928. Subsequently Nägeli[3] demonstrated analogous case with familial occurrence and named this chromatophore nevus. Siemens[4] in 1929 presented a case under the name of melanosis corii degenerativa. His case was admitted by Sulzberger as belonging to this disease. Later Bardach[5] noticed similar cases in twins. The identity of his cases with former cases was eagarly discussed by Siemens and Meirowsky-Leven, and thus attracted the attention of all investigators in the world. The number of this rare condition reported in the literature amounted to 20 including the recent report of Heilesen.[6] The first case in this country was observed by K. Kitamura[7] in 1938 and the second case was demonstrated by Yokoyama[8] at the 250th meeting of the Tokyo Dermatologic Society. The present author observed 3 cases. These are illustrated in Table I.

Discussions.

The characteristic histologic change is the increase of chromatophores in the upper dermal layer. These belong to the endothelial or connective tissue cell origin with negative dopa reaction, and are filled with coarse melanin granules. In the basal cells in the pigmented area, the melanin content is rather decreased and various degenerative changes such as vacuolation in the protoplasm and pycnotic nuclei are noticed. In accordance with the dopa theory, Bloch and Sulzberger explained the pathogenesis of this desease in the following manner. In the normal epidermis the melanin is produced in basal cells and is mostly transported into the upper layer. In this condition the epidermis has become incontinent of the pigment and the melanin granules drop down to the corium and are

TABLE I.

Patient No.	1	2 (Fig. 1)	3 (Fig. 2)
Name	T.	N.	K.
Sex	f.	f.	f.
Age	11 months	7 months	26 years
Consanguinity and familial occurrence	None	None	None
Onset Initial eruption	10 days after birth. Erythematous eruption with intermittent fever.	Existed by birth. Erythematous exanthema.	100 days after birth. Varicelliform eruption.
Symptom	Mortar splash-like pigmentation dark brown in color with undulated zonal arrangement. Zebra-like appearance.	Bluish-brown splash-like pigment patches with marble configration.	Slaty ashy brown pigmentation stellate in form.
Location	Symmetrically generalized.	Symmetrically generalized.	Systematized on the breast, shoulder and flexor surfaces of extremities.
Other Symptoms	Stupid appearance. Slight microcephalia and sudden death by pneumonia.	Congenital nucleal cataracta of the left eye.	Asymmetry of the breast.

phagocyted by the chromatophore. This theory was supported by Miescher who, by injection of dopamelanin to the vitiliginous area, observed that artificial melanin is sustained for a long time in the corium. Siemens accepted Bloch's theory, but at the same time assumed that the melanin production increases. In the material obtained from my third case I obtained analogous histological findings to Bloch-Sulzberger. The existence of argentaffin granules in the basal layer, as well as the negative dopa reaction in the corium, is in favor of Bloch's theory. However, owing to the rarity of this disease, there remain many questions in the etiological points which are to be solved.

In the following I try to investigate this problem chiefly in connection with the study of the nervous system.

(1) The peculiar systematized arrangement of the pigment spots in this disease was already accepted in the discussions on Bardach's case. The participation of the cutaneous nerve is naturally suspected as in cases of systematized nevi. So Voigt's borderline of the distribution of the cutaneous nerve or Blaschko's dermatom arrangement line is proposed to be the site of predilection. The location in typical cases is in arciform romf the breast region to the shoulder and descends along the upper arm,

Fig. 1. Case no. 2.

quite corresponding to the Voigt's line.

(2) In usual dermatoses, the eruption is convex-bordered in accordance with the region of a vasomotor nerve, while in this desease the morphology of the larger eruption is characteristically concave contoured. How is this concave form explained? Here I tried to find some special significans. First of all the reagibility of the vasomotor nerve was examined by histamine scratch test. In the pigmented area the production of the wheal was earlier than the surrounding normal zone by 10–15 seconds. This fact suggests that there exists congenital abnormality.... an interstice either morphological or functional, in the distribution of the vasomotor nerve especially in the constrictor portion. This interstice between the segmental distribution of the vasomotor nerve is the predilection of the pigmentation, hence the concave contour!

(3) Bloch-Sulzberger stated that erythema, swelling and bullous or lichenoid eruption are seen prior to the pigmentation. The existence

Fig. 2. Case no. 3

of the inflammatory stage was observed by most investigators except Naegeli, Moncorps and Nexmand. The significans of this inflammation has not been thoroughly recognized. It is true that the inflammation may stimulate the pigment production as in the pigmentation following teer spread, arsenical intake or actionodermatitis. But it is impossible to contribute such generalized occurrence of inflammation vaguely to the congenital factor only.

On the ground of afore mentioned hypothesis, the author tries to interprete this problem in the following manner.

In individuals possessed of labil autonomic nervous system, there may arise abnormal reactions in part of the vasomotor nerve to external stimuli of the extrauterine environment. These reactions give rise to inflammatory changes espec. in the interstice of vasoconstrictor nerve and thus result in the disturbance of melanin metabolism. The above-mentioned acceleration of the histamine test may have disclosed the remainder of this local disposition.

(4) The pigmentation in this dermatose usually disappears by 4–5 years of age like the mongolian spot. This is explained either as the common characteristics of the dermal pigment cell or as the result of compensation through the correlation of the autonomic nervous system in the loco or through chemical transmission. This is quite corresponding to the observation of Lasser and others that systematized nevi may disappear by itself....the characteristics of the so-called neuronevus.

In my third case as well as in Sulzberger-Praser-Hutner's[9], the pig-

mentation persisted till the youth like the persistence of the mongolian spot in form of the blue nervus.

(5) In all reported cases the pigmentation is most prominent in the perimammillary region. In the case of Sulzberger-Praser-Hutner, the breast of the diseased side was smaller than the other. In my third case the left breast, where the pigmentation was much darker, was by far smaller than the right. I assume than this indicates the retarded development of this merocrine gland through the congenital asymmetric distribution of the trophic nerve, in the sense of the homolateral nervous disturbance. In accordance with this theory I observed two female cases of systematized nevi in the breast region with smaller breast on the diseased side. In another case of a 20 years old woman with hairly nevus in the breast region asymmetry of the breast was noticed. These facts as well as Lewith's[10] similar observation are in favor of my foregoing assumption.

The relationship between melanin production and nervous factor has been established by the experiments of Brücke, Pouchet and v. Frisch on the chameleon and the turbot. In 1932, Parker summarized these results and proposed the theory of neurohumoralism. In human being many investigators found both clinically and experimentally that there exists correlation between melanin production and cutaneous nerve. The author has already reported many observations suggestive of this point. In cases of pigmented nevus, blue nevus, Recklinghausen's phacomatosis and in pigmentary disturbance following perniosis and erythromelalgia and in vitiligo, the role of autonomic nervous system in the pigment production was noticed.

From above the author believes that the foregoing suggestions on the etiology of this disease cannot be a mere phantasy and dare to report on the paper.

Conclusion.

This disease occures in individuals with congenital dysfunction on the part of the autonomic nervous system. These easily react with various grades of inflammatory changes against external stimuli of the extrauterine life. This inflammation results in the disturbance of the melanin metabolism and further in the incontinence of the epidermis. Thus the pigmentation is produced in the characteristic symmetrical and concave arrangement. But the recovery can appear sooner or later when the space is compensated by the correlation of the peripheral nervous system.

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Prurigo nodularis.

By

Susumu Iijima.
(飯島 進)

(From the Department of Dermatology, Faculty of Medicine, Tohoku University. Director: Prof. M. Ito.)

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Recently I observed several cases of prurigo nodularis and obtained some interesting result concerning its etiology, the abstracts of which were already reported in this journal.[1] Here the detailed description of the experiments will be given.

Historical Review.

In the past fifty years this disease was studied by many investigators. Most of them looked upon this either as a variety of urticaria, urticaria perstans, or as a sort of lichenification of Brocq-Pautrier, or as a sort of prurigo, prurigo nodularis. There are some, however, who advocated their own theories and designations.

The oldest conception, the persistence of the wheal was first proposed by Willan and Bateman.[2] F. J. Pick[3] in 1891 reported five cases under the title of urticaria perstans. But some of his cases belong to urticaria pigmentosa and others to what Kreibich later called urticaria perstans simplex, the existence of which is under discussion. The first case bearing certain resemblance with our disease in German literature was reported by Fabry[4] in 1896. Kaposi[5] refered to urticaria verrucosa in his handbook which he regarded as the secondary deformation of the wheal in old individuals. Kreibich,[6] in his article over urticaria chroica, reported two cases of urticaria perstans verrucosa which quite corresponded with prurigo nodularis. He emphasized the urticarial nature of this disease because of the coexistence of the wheal in his case. Hartmann[7] reported similar cases under the title "Ueber urticatielle Hauterkrankungen" and refuted the relationship with urticaria and noted that the eruption was the result of primary pruritus. Thus he ascribed this to a sort of prurigo. Hübner[8] also rejected the urticariogenesis and proposed a new name suggestive of the secondary granulomatous nature, tuberosis cutis pruriginosa, while Fabry[9] in his later article accepted the coincidence with the lichenification of Brocq and advocated a new name, neurodermitis nodulosa.

Rasch[10] introduced Hyde's designation for the first time in the German literature in 1916. In 1920 Ahrens[11] of the Unna's school believed to have found the eczematous origin and proposed another name eczema verruco-callosum nodulare. Vohwinkel[12] recommended the naming lichen obtusus vulgaris and rejected the confusion with urticatia perstans in Wolter's sense. In 1932, Bohnstedt[13] added some new findings by ascribing keratosis verrucosa of Weidenfeld to the group of prurigo nodularis.

In America this desease was more clearly recognized. Hardaway[14] first of all, then Corelet,[15] Johnston,[16] Schamberg-Hirschler[17] reported similar cases without contributing them to no preformed categories except White[18] who accepted the theory of lichenification. In 1909 Hyde[19] gave the name of prurigo nodularis which has since been accepted by almost all authorities in America.

In France Brocq and Pautrier[20] were the outstanding investigators in this field. In accordance with the theory of lichenification, they first proposed the name lichen obtusus corneus, then abnormal lichenification and finally lichenification circonscrit nodulaire was proposed. To them it was beyond doubt that the primary cause was the pruritus and all clinical picture was the result of "grattage". Some investigators, Thibierge, Nicolas and Gate refuted this theory and emphasized that this is a variety of lichen ruber chiefly on the basis of clinical findings. In 1934 Pautrier found hyperplasia of nerve fibers in the eruption and thus attracted the attention of investigators on this point.

Case Report and Discussions.

The author collected 33 cases in this clinic, 14 of which were male and 19 were female patients. The age of onset is shown in Table I.

TABLE I.
Age of Onset.

	under 10	11–20	21–30	31–40	over 41
Own Cases	4	11	12	2	4
Cases in Foreign Countries	5	18	26	20	26

The youngest is 5 and the oldest 71. The season of onset is most often in summer, but there is no definite relationship between the onset of the disease and the season. The site of the eruption in most cases is restricted to the extensor surfaces of the extremities, but in 8 either the face or the trunk or both are involved. Itching of the varing grade is

complained by all and in two the pruritus is the first complaint. Remarkable is the fact that in 12 cases insects bites are blamed as an approximate cause. The disturbance of the menstrual cycle is complained in three, and in two the onset ensuded pregnancy or partuition. In cases from foreign literatures are 23 men and 70 women. The age of the onset, which is shown in Table I, is most often between 21 and 30 and in the class over 41. This shift to the older age may not be significant if we take into consideration the difference of the distribution of the population according to age.

Symptoms. The essentials of the symptoms is the production of nodules. They are disseminated mostly on the extremities, nut or pea sized, semiglobular elevated, verrucous in appearance and brownish in color. The other parts of the body may be involved, and the lesions in those parts may have rather smooth and shiny surfaces and are small in size. Thus the fundamental lesion is the isolated nodulous eruption, and the difference between the papulous and verrucous variety lies chiefly in the difference of location. The grouping of nodules is observed by several reporters, but if we are to admit that lichenified patches may be produced in the papulous type, as is maintained by Dohi[21] and Kitamura,[22] we cannot distinguish it from neurodermatitis. Such instances, if any, can never be admitted into the same category as prurigo nodularis which shows no tendency to plaque formation. Lehner[23] refuted the existence of the papulous variety and enlisted it in prurigo vulgaris.

Pathogenesis. The histo-pathological change in this disease lies both in the epidermis and the cutis. In the former mighty hyperkeratosis, acanthosis and elongation of the epidermal process while in the latter much or less bordered cellular infiltration are usually noted. These never coincide with urticaria or transient edema of the dermal layer. The urticariogenesis of Kreibich cannot be justified. The coexistence of the wheal does not represent the relationship with urticaria any more than the prurigo of Hebra which may start as a wheal-like eruption is a variety of urticaria acuta. The transition of the wheal into the nodule was noticed only by Hieleman. From these it is clear that this belongs to quite different category from urticaria, though the naming of urticaria perstans may be preserved as indicating this particular state. The positive dermographia indicates only the vasolability of the patients and gives no clue to the pathogenesis of the individual eruption itself.

The details of the histological changes vary in a wide range according to the authors. This is chiefly due to the difference of the stage and cannot be significant itself. So it is impossible to draw certain conclusion from some or other histological picture alone. On the other hand the neurom of Pautrier has been regarded as somewhat pathognomonic

by some dermatologists. The nerve change in this disease was first described by Johnston. He found perineural infiltration and suggested that this might be the cause of the intense pruritus. But proliferation or degeneration was not mentioned. Next came Rasch who observed increase of nerve bundles in the infiltrated parts, but further discription was lacking. Ullmann found no morphological changes of nerve fibers. Pautrier stained sections with trichrome method of Masson and found neuroma-like hyperplasia of medullated nerve fibers in the reticulated and papillary layer. His findings were acknowledged by Liebner, Jalomy, Bonnevie, Moncorps and Kitamura and rejected by Bronzini. Recently Perez and Maruri[24] noticed hyperplasia as well as degeneration of nerve fibers by silver impregnation method and assumed allergic origin of this disease. The author excised more than ten nodules and examined with hematoxyline eosine, van Gieson's, Weigert's and Masson's staining, and in some specimen with Seto's modification of Bielschowsky's silver impregnation method. In several sections thick nerve bundles were found embeded in the infiltrate near the epidermis. Some of them show abnormal windings as is shown in Fig. 1. But in other parts apparent normal nerve bundles were also noticed. In contrast sections obtained from Hebra's Prurigo and neurodermatitis, nerve fibers are occasionally found in the midst of cellular infiltrates, but they are neither so thick nor show abnormal windings. Some of the Pautrier's illustration, however, especially the figure of isolated nerve fibers near the borderline to the epidermis is finely imitated in a section from a case of systematized hard nevus with intense pruritus (Fig. 2). In a section of a nodule apparent normal Meissner's tactile corpuscle is involved. This is an interesting finding concerning the endapparatus for itchy sensation.

From above I conclude that Pautrier's neurom cannot be a pathognomonic feature. Its existence depends chiefly on the stage of the eruption (especially in fully developed one). It is quite difficult to find the significans of the nerve proliferation. This can be interpreted in two ways....either primary or secondary. Primary proliferation may not be impossible in view of the fact that nerve fiber can develope with the epidermis as in the case of condylomata acuminata. But it is too hypothetic to assume the viral origin of this dermatose. Blastomatous nature is out of question. If it be the secondary proliferation caused by mechanical irritation, it has common etiology with amputationsneuroma. But it is not always possible to stain the nerve fiber itself in the subepidermal layer even with silver impregnation method, and Pautrier's observation may be only the proliferation of Schwann's sheath. Therefore, more detailed, if possible, serial examination is required to dissolve this problem.

Etiology. In many cases some or other predisposing factors are re-

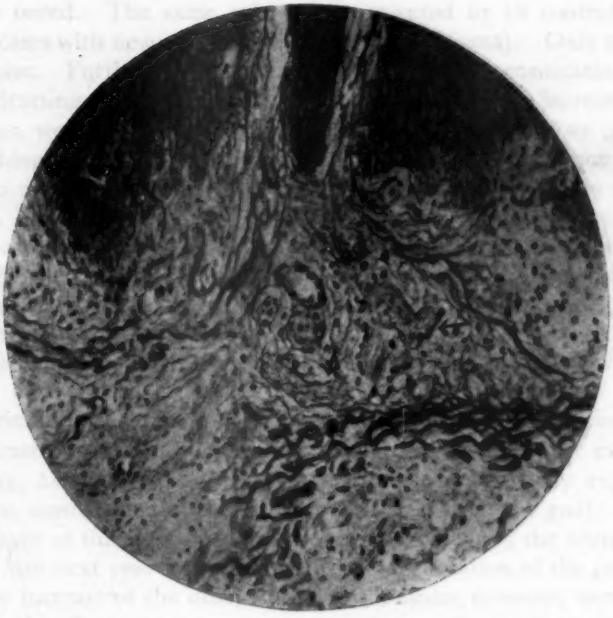

Fig. 1. Winding nerve bundle in the center of a nodule. ×200. ↑ nerve bundle.

cognizable. Disturbance of the endocrine and genitary function are most commonly reported.... such as uterus infantilis (Bohnstedt), dysmenorrhea (Suldberg and others), onset in menopause (Ormsby, Boronsky, Schamberg), in pregnancy (Schönfeld, Vohwinkel), increase of itching during menstruation (Ortynsky, Wise, Fabry), recovery after thyroidectomy (Cole-Driver) etc. Gastrointestinal dysfunction is noticed by Brandt, Epstein, Wile, Lehner and W. Pick. Neuropathia is observed by Lehner, Ketron, Schoff, Pick, Hirsch-Bruck and others. Ortynsky[25] found histological changes in sweat glands and concluded the excretion of menotoxine with the sweat as the cause of pruritus. Similar observation had been made by Netherton[26] previously. In my cases the disturbance of the intermediate protein metabolism is usually noticed. On the other hand many factors affecting the skin from outside are also blamed. Felden mentioned mechanical trauma, Mitchel refered to eczematous changes. In Barnewitz's case as well as Barber's and Vohwinkel's photosensitivity was noted. But there remain many in which no presumptive causes are detected.

Here the author call attention to a hitherto overlooked point—the

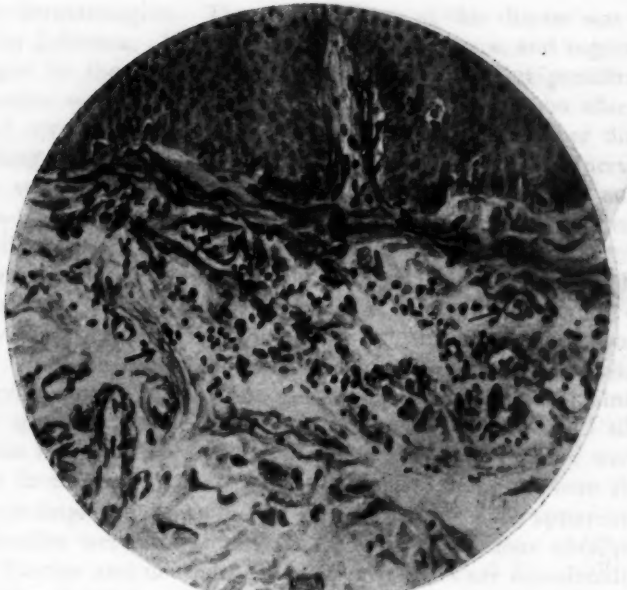

Fig. 2. Nerve fibers near the epidermis. Section from a case of systematized hard nevus.

×200. ↑ nerve fiber.

etiological relationship of this disease with insect bites.

In this country many investigators observed that the eruption often ensuded insect bites. Amongst foreign literatures only Pick, Richter, Schoff, Cole-Driver, and Hirsch-Bruck payed attention to this point. In less than one third of my cases sensitivity to insects bites is complained, and most frequently to a sort of the gnat, Eusimulium equinum Ihnnaeus. This is a tiny insect about 3 mm. in length, frequents in country region in summer time. Its bite gives rise to an extremely itchy wheal-like-eruption about 1 cm. in diameter with characteristic central hemorrhage.

Sensitivity tests were made in the following manner. According to the method of Storm van Leeuwen, 1% suspension of the insect in a solution containing 0,4% $NaHCO_3$ and 0,5% NaCl is prepared. After 24 hours it is filtered and the filtrate is sterilized twice by 56°c for 30 minutes and diluted beginning from 1: 100. In five cases out of seven this diluted extract gave positive reaction by intracutaneous method. In all positive reacting cases sensitivity to this insect was stated, while in the negative cases susceptibility to mosquito bites was stated though mosquito extract

was not tested. The same extract was injected to 19 control patients (mostly cases with nevus pigmentosus and hemangioma). Only two reacted positive. Further trial was made to obtain desensitization in five cases. Starting from 0,1 cc. of 1:100 solution, gradually increasing doses up to 1 cc. were given hypodermally every other day. In four cases relief from itching was procured after several injections and cutaneous eruptions began to vanish graduàlly. In two cases given more than fifty injections the skin has become entirely clear of eruption leaving pigmented and slightly atrophied spots. They are still under observation. In the first case, a 20 years old girl, no recidive has since occurred, in the other, a 22 years old male, the eruption has almost cleared except for occasional appearance of tiny papulo-vesicles.

This extract showed negative Millon's, biuret and xanthoprotein reaction and positive Molisch's and Liebermann's reaction.

These findings indicates that there exists specific sensitivity in patients' skin against this extract. Liebner,[27] though the details of the experiment is lacking, assumes the allergic origin of this disease. My experiments reveal the sensitization of the skin through bites from the gnat. Another fact in favor of this hypothesis is flare up phenomena of the eruption after another bite next year in one case and the aggravation of the pruritus by too hasty increase of the dosage. Antihistaminics, however, were without effect on this disease.

Then to what type of allergy does this disease belong? If it be allowed to classify skin allergy into epidermal and dermal allergy, it is proper to enlist this in the latter because of the positive intracutaneous reaction, as in the cases of hypersensitivity to beas or other insects. The problem whether the itching is the first manifestation of the allergic reaction or the eruption precedes the itching is difficult to answer. Anyway the verrucous appearance is chiefly due to the result of intensive scratching and especially to the characteristics of the location. The difference from normal and other types of abnormal lichenification is explained by assuming that the allergic reaction takes place repeatedly at the site of the eruption and that thus the itching is restricted to the individual eruption.

Naturally it is difficult to ascribe all the cases of prurigo nodularis to the sensitivity to insects bites only. For example Miura observed sensitivity to milk in his case. And the role of insects bite must be carefully estimated. I observed an interesting case in this point. In a healthy 26 years old man, who had insect bite on the lower extremities in summer, a solitary nodule developed at the lower end of the leg in autumn which in all detail coincided with prurigo nodularis eruption. Insect reaction was positive. By next spring this eruption had vanished without any therapy. This reveals the existence of an abortive form or prurigo no-

dularis mitis.

Thus I conclude that prurigo nodularis is mostly a sensitivity dermatose in this country. The sensitivity, however, differs in a wide range in individual case. And in cases with irrecovorable predisposing factors the disease may persist throughout the life if not treated radically, while in slight cases it may disappear by itself.

SUMMARY.

1. 33 cases of prurigo nodularis are reported.
2. In the histological study, the existence of Pautrier's neuroma is verified, the significans of which remains still unanswered.
3. In the study of the etiology, sensitization to insect bites is noticed. This is certified by intracutaneous reaction and further with therapeutic results with the extract.
4. The instance of the abortive form is observed.

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Biochemical Studies on Carbohydrates.

CXL. On Mucoitin from Vitreus Humor.

By

Tyuiti Isikawa.

(石川 忠一)

(From the Medico-chemical Institute, Tohoku University, Sendai. Director: Prof. H. Masamune.)

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Meyer and Palmer[1] said "hyaluronic acid" from vitreus humor and the polyuronic acid from umbilical cord are very similar, but did not conclude the identity of these acids. Their preparations of the acids possessed noticeably diverging optical rotatory powers [hyaluronic acid, −51° (25°C); funis polyuronic acid, −65° (25°C)], despite of concordant analytical figures of components. Later on, Suzuki[2] obtained purer preparations of the acids, which analyzed practically same, but differed in the behaviour towards Ehrlich reagent, vitreum "mucoitin" immediately giving the colour reaction (tested on the vitreum mucin). $[\alpha]_D$ of the vitreum substance was −72.4° (15°C) nearly in accord with that of the funis substance (−70.9° (18°C)). More recently Osaki and Hasegawa[3] prepared an authentic specimen of the funis "mucoitin" whose $[\alpha]_D^{13}$ was −64.9°, a value smaller than that of Suzuki's corresponding specimen.

To clarify the exact relationship between the two polyuronic acids, the writer again tried to obtain a pure preparation from the vitreus humor (bull) by employing the procedure in the former account[4] combined with following electrodialysis. The product not only analyzed just similar to the one of Osaki and Hasegawa from umbilical cord but had resembling specific rotation ($[\alpha]_D^{22}=-65.7°$) at that. Its iodine use, wherefrom the molecular weight may be evaluated, also agreed with the finding of Osaki and Hasegawa on the funis mucoitin. The identity of the two acids was thought thus established. To the same conclusion pointed the properties of its basic disaccharide unit, which was separated as heptacetyl methylester as from funis mucoitin (Cf. the former work[4]). Amino sugar in the vitreum mucoitin was identified with glucosamine as by Meyer and Palmer.

Experimental.

Vitreum Mucoitin.

Vitreus humor of bull eyes was strained through a gauze and precipitated with 5 volumes of alcohol and dried. 3 g. of the substance thus prepared and dried (The biuret, Goldschmiedt, and direct and indirect Osaki-Turumi reaction were given. Molisch, negative.) were taken and crude mucoitin was separated as from funis mucin by shaking the solution of the material in dilute baryta with formalin with following centrifugation, condensation of the supernatant liquid and precipitation with alcohol. 1.5 g. substance was given. It was removed from a water-insoluble part and further from the contaminants coagulable by a chloroform-amylalcohol-mixture (Here the organic solvents were mixed in the proportion of 5:1 by volume). As to the details, cf. the former account.[4] The product weighed 0.6 g. It was transparently soluble in water. Molisch, biuret and direct Osaki-Turumi test resulted in negative contrary to the positive indirect Osaki-Turumi (for acetylhexosamine) and Goldschmiedt and Neuberg-Saneyoshi (for hexuronic acid) test. Hot Fehling solution was reduced by a large amount of the substance, but cold Tollens solution was not. Nitrogen 3.19%; glucosamine 38.19%; ash 10.09%.

With the aim to reduce ash, it was dissolved in a smallest possible quantity of water, 10 volumes of 98% glacial acetic acid added (at first in drops with kneading and afterwards in larger portions), whereby precipitation occurred, and shaken. On the centrifugate were the operations repeated twice more and finally the residue washed with alcohol and ether and dried. Since, however, the substance obtained left still no small amount of ash on burning, it was subjected to electrodialysis (Pauli apparatus, 200 volts) until a renewed water in the outer cells showed no shift of pH (It took three days). The solution was precipitated with alcohol after vacuum distillation to a small volume, and washed and dried as before. A fluffy powder with vitreus luster was given, that gave analytical figures in Table I and had the optical rotation:

$$[\alpha]_D^{22}=\frac{-0.415\times 100}{0.6315\times 1}=-65.71°.$$

No mutarotation happened. From the iodine use (7.47 mg. substance reduced 0.0982 cc. 0.02 N iodine solution) the molecular weight is calculated at: $0.00747\times\frac{100}{100-0.62}:x=\frac{0.0982}{1.06}:100000.$ $x=8110.$

Heptacetylmucosinmethylester.

It was prepared in a manner also reported in the former account.[4]

TABLE I.

Analysis of Vitreum Mucoitin.

Analysis	In per cent	In equivalents per equivalent weight
Nitrogen*	3.29	0.99
Glucosamine	42.49	1.00
Glucuronic acid		
By orcinol m.	46.20	
By indole m.	45.97	
Mean	46.09	1.00
Ash†	0.62	

* Micro Kjeldahl. † Micro analysis.

3.5 g. of vitreum mucoitin obtained as above (a product after the operation with the chloroform-amylalcohol mixture) were hydrolyzed with oxalic acid and 1.5 g. of mucosin oxalate (yellowish brown and hygroscopic) were given. Mucosin methylester hydrochloride was synthesized starting immediately with mucosin oxalate (not with free mucosin). 0.6 g. of the material gave 0.5 g. of a yellowish, brown, very hygroscopic product, which in turn gave 120 mg. of crude heptacetyl-mucosin methylester (needles, F.P. 125°C). Recrystallized from boiling alcohol this showed the constant melting point of 130°C and the mixed melting point test with the corresponding product from funis mucoitin showed no depression.

The substance was readily soluble in acetone, chloroform, ethylacetate and pyridine, quite soluble in methanol, ethanol and benzene, and insoluble in ether, petroleum ether and cold water. Positive indirect Osaki-Turumi reaction of acetylhexosamine and Goldschmiedt and Neuberg-Saneyoshi reaction of hexuronic acid were shown. Fehling (hot) and Tollens (cold) reagent were reduced.

Quantitative analysis:

Crystalline water.

32.87 mg. substance lost 2.51 mg. of water at 100–110°C under reduced pressure.

$C_{12}H_{13}NO_{10}(CH_3CO)_7(OCH_3)+3H_2O$. Found, 7.64%; calc., 7.53%.

Molecular weight from iodine value (Bergmann and Machemer).

17.77 mg. substance, 6.257 mg. iodine used (iodine value 27.74).

$C_{27}H_{43}NO_{21}$. Found, 721; calc., 717.

N (micro Kjeldahl).

11.19 mg. substance, 1.58 cc. 0.01 *N* acid neutralized by distilled NH_3.

$C_{27}H_{43}NO_{21}$. Found, 1.98%; calc., 1.95%.

CH_3CO.

9.62 mg. substance, 9.42 cc. 0.01 *N* thiosulfate solution used (4.0506 mg. CH_3CO).

$C_{13}H_{16}NO_{11}(CH_3CO)_7+3H_2O$. Found, 42.11%; calc., 41.98%.

Optical rotation in chloroform.

$$[\alpha]_D^{15}=\frac{+0.33\times 100}{2.240\times 1}=+14.73^\circ \text{ (no mutarotation).}$$

Identification of Glucosamine as Hydrochloride.

0.5 g. of vitreum mucoitin (from the same batch as immediately above) was hydrolyzed as by Hiyama[5] on the principle of Levene. The filtrate of the hydrolysate was bubbled with H_2S gas and after another filtration, distilled in vacuo to syrupy consistency. To the residual 3 drops of conc. HCl and 2 cc. of methanol were added. And to the solution acetone was added in small portions till faint turbidity. In a short while, crystals in colorless plates began to appear. After standing in a refrigerator overnight, the deposits were collected on a suction funnel. The yield here was 90 mg. The substance was taken up in a small quantity of water and again crystallized out similar to above. The final product began charring at about 180°C and did not liquefy even at 250°C. Soluble in water and methanol but neither in either nor in ethanol nor in chloroform. It reduced hot Fehling solution and gave the positive Elson-Morgan reaction. Chloride ion was detected.

Nitrogen (micro Kjeldahl).

3.02 mg. substance, 1.390 cc. 0.01 *N* acid neutralized by distilled NH_3.

$C_6H_{13}NO_5\cdot HCl$. Found, 6.44%; calc., 6.50%.

Optical rotation in water.

18.65 mg. substance was taken up in water and filled up to 1 cc. Mutarotation occurred as below, which indicated it to be α-isomer of glucosamine hydrochloride Temperature 15°C.

Time	α	$[\alpha]_D^{15}$
min.	deg.	deg.
5	+1.84	+98.7
10	+1.78	+95.4
30	+1.65	+88.5
60	+1.60	+85.8
60×2	+1.52	+81.5
60×4	+1.40	+75.1
60×6	+1.35	+72.4
60×8	+1.35	+72.4
60×24	+1.35	+72.4

Through the Grant Committee for Scientific Researches was furnished from the Education Department a grant which enabled us to carry out this work.

H. M.

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Silver Impregnation for the Nervous System Being Used in Our Laboratory.

By

Hachiro Seto.
(瀬戸八郎)

(From the Anatomical Laboratory of Prof. H. Seto, Medical Faculty, Tohoku University, Sendai.)

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To be most regarded as a matter of grave affair in the study of histology of nervous system is that the studying method should not be incomplete. Because any natural and real views could never be effected, if there was a fault in the staining. As our method ought to be compared with the diagnosis before the treatment of the clinical authority, it is beyond dispute how important it is.

That the histology of nervous tissue has fallen too much behind that of other tissues in development depends upon such a fact, that discovery of its successful ideal staining has not appeared because of its extreme difficulty. In order to clear the definitive signification of the nervous tissue one must try in earnest to represent not only its essential elements, "Neurofibrils", positively, but also at the same time its accessory elements and the other various tissues surrounding so specially and beautifully, that the evident difference between both of them might be easily examined through the microscope. The so-called ideal staining is namely such a method, that all kinds of tissues in every section can be stained clearly at the same time. It is therefore considerably difficult to originate such a brilliant one.

The silver impregnation of Bielschowsky which followed Cajal's one was the very first step to the ideal staining. Bielschowsky's impregnation has since then given birth to the various modifications by many authors. The impregnation modified by Stöhr since 1928 has been above all extraordinarily so excellent, that he has succeeded with his pupils in discovering many new views. His great difinitive contribution to the histology of nervous system has been commended universally by the world.

I had in 1932–33 an opportunity to study the nervous histology under Prof. Stöhr at the Anatomy of University Bonn a. Rh. in Germany, learned his silver impregnation directly from him and was impressed greatly by its superiority. After returning to Japan I have still now tried so earnestly

to make the Stöhr's method more ideal, and succeeded before several years in finding out such a excellent one, as follows. Its excellency became already the object of the extraordinary admiration of Prof. Wasano, Kyushu University, and I am also confident of the best one of the staining formulas for the nervous system, which self and others together should recognize.

1. Operation. To fix use 10 per cent neutral formol. For this purpose one needs in the first place to neutralize the commercial formol solution by adding calcium carbonicum precipitatum in proportion of 1/12. At the moment of using it, dilute this mother solution to 10 per cent with distilled water, taking its upper solution already cleared. The materials remain almost more than half an year in this fixer, being sectioned with the freezing microtome afterwards. The sections remain further in the fixer and when desired to stain only such a few pieces as desired are taken out from the formol solution.

When one will hurry in making preparations, section the material with the freezing microtome after fixation for 1 to 2 weeks and continue further to fix, one could then acquire good results after 2 or 3 months.

30μ to 40μ is suitable in thickness. If the sections are too much thin, the cubic observation of the pictures of tissues is not effective.

2. Operation. For the operation of the sections from now, use a glass stick bended ahead hook-like. The number of sections to be stained are suitable for the beginner about 7. The sections taken out from the fixer remain now for a short time in distilled water.

3. Operation. Then place for 1 to 3 days in 20 per cent silver nitrate fluid filled in a glass plate of good quality. This silver solution is for about 20 days useful.

4. Operation. After immersing for 10 to 20 seconds in distilled water one must turn to the 3. operation. So one should prepare the 5. operation before passing to the 4. operation from the 3. operation.

5. Operation. One must be ready to make 200 to 300 cc. of 20 per cent neutral formol, which should be made only by deluting the mother neutral formol by all means with running water. This formol solution is then divided into 4 to 3 plates. The sections placed in distilled water in the 4. operation are transferred now by turns from the first plate to the last. Here is reduced a part of silver, producing milky precipitates. This precipitation is carried in the first plate most vigorously, decreases gradually in the 2nd and 3rd plate and disappears almost entirely in the last plate.

It is interesting that the disappearance of the precipitation in the last plate is limited only for the materials fixed with neutral formol, while the phenomenon of this kind does not appear on the sections fixed with acid formol, that is to say, the white precipitates do not disappear in the later

instead of renewing water formol solution even more than 10 times, of course without effecting good results in staining afterwards.

6. Operation. Next procedure consists in transfering sections to ammonical silver solution just after washing with running water. As the washing time in the water is very short, one must set about making a ammoniacal silver solution as soon as one has finished the 5. operation.

Formula of ammoniacal silver solution. Set a suitable amount of 20 per cent silver nitrate solution into a glass tube kept clean with distilled water, then drop into it ammonia powerful as possible suitably. The amount of dropping ammonia is not settled constant throughout the four seasons because of having a intimate relation with the chamber temperature, but it is kept in general in 1/5 proportion for silver fluid. In the limitting part between silver fluid and dropped ammonia appears a brown line. Shake the tube up and down after stopping a cork, then this mixture becomes brown. If one could operate, that the precipitation might be colored very poor and weak yellow, such a operation is most convenient, because the mixture becomes immediately clear by adding further only a drop of ammonia. It is the most ideal formula of ammoniacal silver fluid. If the precipitates are produced abundantly and all the fluid has become dark brown, one must continue the dropping of ammnoia and try to clear it with a last drop.If, on the contrary, the mixture has become transparent by the first shaking, one can make clear it by means of dropping ammonia after producing brown precipitation of the minimum by adding a little amount of silver nitrate.

It would be especially noticed, that the formula of ammoniacal silver solution must be finished as rapidly as possible. The time the longer, the staining results afterwards the worse.

The ammoniacal silver fluid prepared in now placed in a watch glass.

The subject returns here to before. The sections in the last plate filled with 20 per cent neutral formol, which was diluted with running water, are transferred by turns to running water in a plate. This procedure consists really in removing the affinity of connective tissue with silver. It deprives namely the connective tissue of its silver affinity, which appears in general as in the same grade as for the nerve elements, and contributes to make easy very much the distinction between the connective tissue and the nerve elements which stain a dark violet color. This procedure requires about 30 seconds, but the time in running water has to be controlled by various conditions.

Next taking out the sections by turns from the running water and blotting the water with filter paper, transfer to the ammoniacal silver solution prepared in a watch glass, then they are stained gradually a reddish brown color. This staining should be finished by all means just in

about 10 minutes. It is here very important not to hurry the staining but to proceed gradually. When the speed of staining is too fast, silver precipitates stick so strong to the sections, that the results are not good, when it is too slow, they are more effectless.

By the way, the proceeding speed of staining is in proportion with the chamber temperature. When the temperature is high, the sections stain easily, when it is low, they stain hard. Especially in the case of the later therefore, the warming of the watch glass with ammoniacal silver is decidedly necessary.

This warming formula is to be acted with following standard. When the chamber temperature is nearly 0°C, the watch glass is warmed by boiling water filled in a plate, when about 10°C, it is warmed by warm water, then gradually by boilling water, when about 20°C, one needs any warm water. In the middle of summer the staining is possible without warming, but even in this time it effects much better to use lukewarm or warm water.

By Stöhr's method one observes often the sections in the watch glass, with ammoniacal silver fluid through the microscope, in order to examine how they are staining. But it is unnecessary in our method to do it by experience: after observing with naked eyes the best staining tincture of the sections, in other words at the time, when they seemed to have stained most convenient—as it is possible to get the standard of the staining condition by examining the preparations of the sections, which already completed, through the microscope, so we have only some practices before at least 2 or 3 times by using the sections of the same material—we transfer to next operation, merely picking them from the fluid without using microscope.

7. Operation. Next by Stöhr's method the sections are transferred to 2/8 diluted ammoniacal solution with distilled water. But the fluid is still too strong. If we want to see it, it must be more diluted to about 1/3. This procedure consists in removing the tumultous precipitation of silver. In 20 to 30 seconds the sections are immersed in a dilute solution of acetic acid, in order to acidify just a little. But it was cleared that the operation of this kind does more harm than good, especially acetic acid stains the sections a bluish tinct so often. Therefore instead of using these reagents, we have succeeded in acquiring better result by means of carrying the sections successively in 2 plates filled with distilled water.

8. Operation. Next the sections are transferred to a 1/1000 or 1/5000 solution of gold chloride. Gold chloride truely should be used as a solution as dilute as possible, that is the best showing only a weak yellow tinct. Because if it is too strong, it acts upon the sections so rapidly and vigorously, that not only the nerve elements, but also the other tissues stain univer-

sally a bluish black color. When the sections are stained so convenient in about 3 to 4 hours.

9. Operation. One immerses them in 15 to 20 per cent natrium hyposulfite solution, after washing them in distilled water. This fluid is also for about 20 to 30 days useful enough. Natrium hyposulfite removes black brown stained excess of gold from the sections and gives them now a beautiful reddish violet color. After about one minute.

10. Operation. Wash the sections with distilled water 2 times, dehydrate gradually in 70, 90, 95 per cent alcohol (often absolute alcohol is used too), transfer to Carbol-Xylol and mount in Canada Balsam at last.

On the preparations thus stained there is no anxiety for changing their color in future, on the contrary it seems that they are increasing their clearness day by day.

By our staining method not only the nervous elements are able to be stained to extremely fine neurofibrils a beautiful black violet tinct, but all the other tissues also take every their special colors. Therefore the nerve innervation of every tissue can be made so clearly.

At the end in the case of trying to use the impregnation for the organs which contain bone tissue, one must decalcify in the first place before silver staining. The decalcifying method in this case, of course, must be somewhat modified from the usual one. The principle of this modification, that is to say, depends upon not to remove the material from the formol fluid. Because if the material is apart from the formol solution the silver staining afterwards never gives us satisfactory results.

This special decalcifying is just carried out as follows. For the purpose of decalcifying is used the mixture of 10 per cent neutral formol 49 cc., 5 per cent nitric acid 49 cc. and acetic acid 2 cc. When one uses the mixture of this formula, the decrease of the impregnations power of silver does never take place. After decalcifying one washes the material with running water for 24 hours, next for 5 to 7 hours in distilled water (several times renewing), then transfers it to 10 per cent neutral formol again and sections it by using the freezing microtome after 2 or 3 days.

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A Simple Benzidinepaper Test for Occult Blood Examination.

By

Kinshi Mitsuki and **Motoko Uyesugi.**
(参木 錦司)　　(上杉 もと子)

(From the Medical Clinic of Prof. T. Kurokawa,
Faculty of Medicine, Tohoku University, Sendai.)

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The chief tests of occult blood, that have been known hitherto, are guajacticture test, (Schönbein-Almen, Weber van Deen), pyramidon test Thevenon et Rolland), benzidine test (Adler and Schumm) etc.

The results of these tests are different from each other in reactional sensitivity and in positivity, as are known: Moreover, when these tests are carried out, test tubes, considerable quantity of reagent, and much trouble needed. So the occult blood test is apt to be neglected by practitioners, who are leading very busy lives, on account of scantiness of time and reagent, though they are aware of the importance of it for their physicians activity.

We regretted this point greatly, and thought that it would be very useful and helpful for us, if a new method of the occult blood test was found out by which the test could be carried out more simply, more cleanly than by the former methods.

After much thought and many experiments, we succeeded in inventing a kind of reactional paper, that is OCCULTOR. By using it in our clinical test, we could get the same result as by the former methods without so much time and trouble. It is the purpose of this paper to evaluate the results of the ordinary laboratory procedure in comparison with ours. So our new method of the occult blood test will here be made public.

Materials and Methods.

1. The making way.

Put 100 cc. of 6 per cent alcoholic benzidine solution and about 10 grm. of soluble starch into a mortar, and make them pasty by grinding down and mixing together. Apply this paste on a large sheet of filter paper evenly and leave it in a room for a day or two, so that it may dry by the temperature there. Then cut it into small pieces one centimetre square. This is the OCCULTOR.

2. The testing way.

Put a piece of the OCCULTOR on a clean sheet of glass, with the pasted side upward; put on the pieces a very little of the substance to be examined (for instance feces), and spread it on the paper with a glass-rod, giving a slight press. Fall on it, a drop of the first reagent (glacial acetic acid) from a dropping bottle, and then drops of the second reagent (hydrogen peroxide) on it soon after.

3. The deciding way.

According to the length of the time required between dropping of the second reagent and the appearance of surrounding colour-changing (a deep blue colour: Indigo) of the feces on the paper, the decision is made as follows:

within 30 seconds .....(卅)
within 1 minutes .....(廾)
within 3 minutes .....(+)
3 minutes and more .....(±)

When there appears no change of colour on the paper after three minutes, it is decided to be negative. But some times (+) appears according to the kinds of food and medicine the tested patient has taken. So we think it is (卅) and (廾) that can safely be relied upon as the results of our new method applied to feces-test.

The parallel tests of the original methods and our new one were carried out with all the in and out patients of our internal department of the Tohoku university hospital and it proved that our methods brought about the same results as the former ones, with less time, less trouble, and less cost than the latter. So at the internal department they have adapted our new method instead of the original ones.

As for the results of the comparison tests between the new and old methods our reactionary paper shows true positive blood reactions in dilutions up to 1:100,000 in the soluble hemoglobin compounds, and it is, on the whole, in accord with the guajactincture test. It means that its reaction is stronger than by the pyramidon test, and weaker than by the benzidine test.

These points are very important in deciding positivity and we do not hesitate to decide that it is absolutely positive when our reactionary paper-OCCULTOR shows (卅) and (廾). By using this paper method, the test can very easily carried out, especially when at the mass-examination of occult blood or at the patient's house we visit. It has proved that this test apper can safely be kept and used for at least one year after its made.

SUMMARY.

Here we have made public our new method of the occult blood test.

This new method can be carried out more simple, more economically, and more clearly than by the original methods and it brought about the same results as the original benzidine test. The maximum sensitiveness of our method was found to be 1:100,000 with hemoglobin.

We add that the details of this paper was already made public by Drs. Mitsuki and Uyesugi at the 141st Conference of the Tohoku Medical Society in March, 1946.

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On the New Duodenal Tube.

By

Kinshi Mitsuki and **Yuji Shirane.**
(参木 錦司) (白根 雄二)

(From the Department of the Internal Medicine, Akita Prefectural Hospital, Akita. Director: Dr. K. Mitsuki.)

(Received for publication, September 19, 1950)

The duodenal tube by Meltzer-Lyon's test is, needless to say, very important and necessary for the medical examination and treatment of the various biliary diseases. The tube which has been used till now in the method, chiefly consists of the olive which was invented by Einhorn and was a little improved later (for instance the one devised by professor Matsuo of the Medical Department of the Kyoto University). Though any great inconveniencies are not felt in using the tube, it is obvious that it would be still more disirable if we could reduce the time required to reach the destination, especially concerning with many patients or with the outpatients who must be dealt with in limited time.

With the object of fulfilling this desire, we have devised a new kind of the duodenal tube. By using our new one we have had a very satisfactory results in our daily examination as well as treatment. So we will make public the comparison between the former tube and our new one.

Construction of Tube and Results of Experiment.

1. The construction of our new tube.

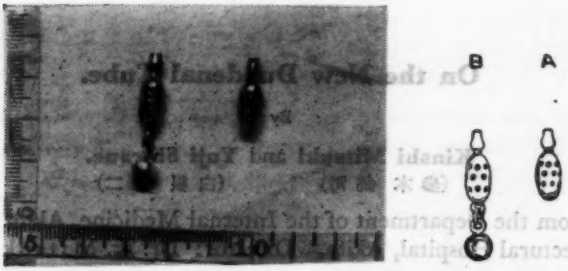
Our olive is an oval ball 12 mm. in length and 5 mm. in breath and is empty inside. It has 18 round holes, 1,5 mm. in diameter on the surface. At the bottom of the olive there hang three links in chain each of which is 2 mm. in diameter, and at the third link there hangs a silver plated small ball, which is of 5 mm. in diameter and of 1.6 grm. in weight. While the classical olive has the weight of 1.3 grm., our new one is 3 grm. together with the silver ball. (Refer to Figure 1)

2. The way of swallowing the tube.

As it is quite the same with the former one, the explanation is omitted.

3. The comparison test between the former tube and our new one. First, we carried out the test of time with one and the same patient whose stomach are recognized in function as normal. When the patient

Fig. 1



A. The original duodenal tube. B. Our new duodenal tube.

has swallowed down each of the rubber tubes at each time, as far as about 40 cm. measured from the incisor of the patient, we had the patient lie down on the bed, with his right side down, and let the tube go down by itself. At the same time are esamined, according to time, the position of the olive through X-ray and the change of quality of juice taken through the tube.

For example, we mention one case in the next space.

When one minute and 58 seconds have passed after 40 cm. of the rubber tube was swallowed down, both of the olives were found about at pyloric antrum. When 15 minutes and 30 seconds have passed the olive of our new tube had already reached the genu superius of the duodenum, while the former olive had not yet passed the pyloric region. When 21 minutes and 23 seconds have passed, our olive got to the pars descendens, and we could take the bile which streamed through the Vater's papilla, though the original olive had just passed the sphincter of the pyloric region. Thus this experiment showed that our new tube could be swalled in far less time than the former tube.

We carried the same test with 20 patients who were in the hospital of our internal department suffering from the diseases of bile ducts system, and the results of the tests, as the Table I. shows, made it evident that the time which our tube needs in reaching the pars descendens can be reduced to about one-third of the time which is required for the former tube.

Discussion.

We think there are two reasons why our new tube can reach the place aimed at in so less a time. One reason is the weight of our olive, which is about two times of that of the former olive. This weight makes it easier for our olive to go down along the lesser curve of the patient's

TABLE I.

Case No.	Name	Sex	Age	Diagnosis	Kind of tube	Time (minute)
1	K.H.	♂	23	Cholecystopathia	O N	50 21
2	T.M.	♂	29	"	O N	60 30
3	R.H.	♂	33	"	O N	60 50
4	T.K.	♀	38	"	O N	180 120
5	W.K.	♂	28	Choledocusstone	O N	180 60
6	T.K.	♀	48	Cholecystopathia	O N	180 120
7	T.K.	♂	32	Jaundice	O N	120 180
8	Y.T.	♀	36	Choledocusstone	O N	150 180
9	S.K.	♂	20	Liver-swelling	O N	160 110
10	H.S.	♀	27	Choledocusstone	O N	100 70
11	K.T.	♂	24	Jaundice	O N	180 40
12	K.H.	♂	22	Cholecystopathia	O N	90 70
13	T.T.	♂	53	Choledocusstone	O N	30 10
14	T.K.	♀	29	"	O N	120 80
15	K.S.	♂	61	Liver-distoma	O N	120 70
16	K.O.	♂	24	Jaundice	O N	180 110
17	T.S.	♂	52	Choledocusstone	O N	120 60
18	K.T.	♀	36	Cholecystopathia	O N	110 60
19	K.M.	♀	53	"	O N	120 180
20	G.M.	♂	46	Choledocusstone	O N	120 60

O=the orginal tube.
N=the new tube.

stomach. The other reason is the silver small ball attached to the olive. Because the sphincter of the pyloric region tries, as soon as possible, to excrete down the ball as foreign body, the olive which is connected with it can also pass the sphincter as soon.

Summary.

We have devised a new duodenal tube and by using it we have been able to take the dudenal juice with the time reduced to about one-third of that the original one required.

We believe that our new tube will play a very effective part in the medical examination and treatment of the various biliary diseases.

The details of this paper was already made public by Drs. K. Mitsuki and Y. Shirane at the 12st meeting of the Thoku Session of the Japanese Gastro-Enterological Society in June, 1950.

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Experiences on Labyrinthine Surgery.

First Report.*

By

Yutaka Tsuiki.
(立木 豊)

(From the Institute of Oto-, Rhino- and Laryngology, Tohoku University, Sendai. Director: Prof. Y. Tsuiki.)

(Received for publication, September 25, 1950)

Ménière described a new disease of the labyrinth in 1861 establishing first an intimate connection between the ear labyrinth and virtigo. Although this disease was in commemoration of his epoch-making work called by his name, the later progress of the pathology of the labyrinth seemed to deny the real existence of this disease but to point only to a syndrome. However authors in these 20 years incline to again accept it as an individual disease of the peripheric labyrinth, although, concerning the nature of the disease, mere speculations have been expressed without any convincing proof.

In 1927 Portmann[1] described a method of operation for the relief of aural virtigo. He opened the saccus endolymphaticus, reaching the fossa endolymphatica through the mastoid. In these 2 years the writer had several occasions to practise the operation for the relief of virtigo and wishes here to report the results of the operation with some considerations regarding the nature of the disease.

Case Reports.

Case 1. A man aged 22 came to us with complaints of tinnitus, hardness of hearing in the right ear and attacks of virtigo. The history showed that the first attadk occurred when 16 years old. The onset was sudden without any suspicious cause. He had virtigo, nausea and vomitting. He could not stand up. 10 days before the first attack he had tinnitus in the right ear. Since the first attack hearing began to diminish in the right ear. The first attack lasted half a day. He underwent conservative treatment for 4 months in the Policlinic of Internal Medicine. Deafness was gradually relieved untill he could hear a telephone, but tinnitus was not improved and increased from 1941. In Sept. 1943,

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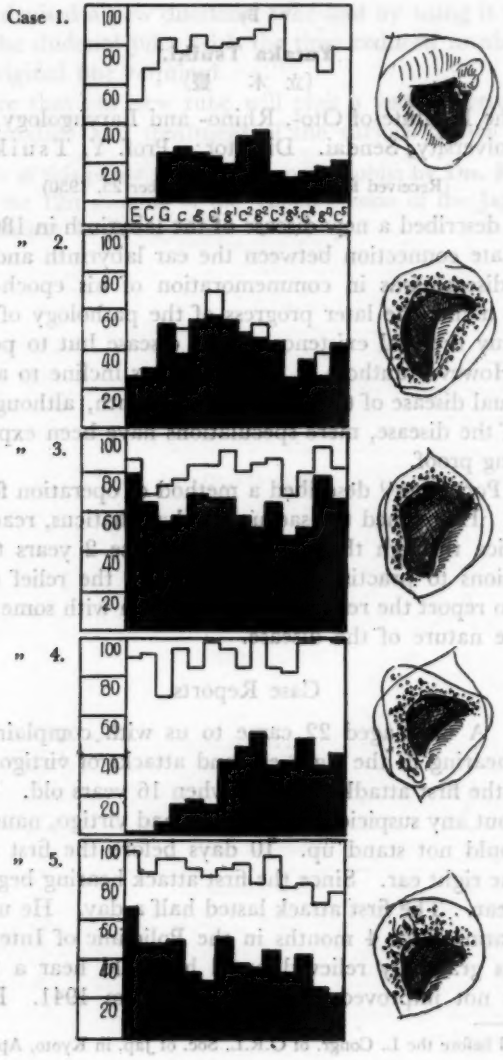
during sleep, virtigo attacked the patient as before. After that he had attacks on and off, and when attacks were severe, he lost his consciousness.

Case 2. A man aged 36 had a sudden onset of virtigo in May 1945, when he got out of bed at night. The first attack lasted several days and



during the attack he remained in bed. In May 1946 he had the second more severe attack than the first. In the beginning of July the third attack set on. This was most severe and continued till the 14th of the month when he was admitted. Extreme virtigo was accompanied by disturbance of equillibration and at first he was unable to stand up or to foot. A few days before admission he became able, it supported, to foot. He was dizzy almost every day. The sense of movement did not disappear even in bed.

Case 3. A man aged 21, had a sudden attack, when at work, of virtigo associated with nausea and vomitting. After 4 days from the first attack happened the second. In this case also nausea and vomitting accompanied it. In two weeks nausea and vomitting were relieved, but heaviness of head and vertigo did not reduce. The virtigo was accentuated by sudden movement of the head.

Case 4. A married woman, a teacher of an elementary school, aged 34 had virtigo and headache since Nov. 1947. Owing to attacks she was prevented from doing her work and confined in bed. In Nov. 1948 she visited the Gynæcology Department, but no change was found and she was sent to Internal Medicine and from there to us. There aws a history of previous middle ear-disease (left ear), but this had now cleared up.

Case 5. A married woman aged 41 had first tinnitus in the left ear without any suspicious cause in Aug. 1948. Towards the end of the month she had a severe attack of virtigo with a sense of rotation of external objects toward left. This attack lasted 4 hours, but untill admission in Apr. of this year she had attacks eight times. Since Nov. of the last year she was dizzy almost every day and confined in bed. She took Luminal-tablets every day. Since a month before admission she had tinnitus in the right ear.

Results of the Operation and Discussion.

The results of the operation can be resumed as follows;

1) The retroauricular wound healed primarily in a week or 10 days.

2) In virtigo the writer obtained satisfactory results in all instances. Only in advanced cases the complete disappearance of virtigo took some time after the operation.

3) The disturbance of equillibration recovered in parallel with virtigo. For instance in the second case the progress after the operation was very favorable. Virtigo disappeared and the patient, who had been confined to bed, became able to walk freely.

4) In all cases heaviness of the head disappeared immediately after the operation.

5) Some patients complained of tinnitus or its temporary increase

even after the operation, but this symptom disappeared also with time.

6) The audibility, when its interruption was not so advanced, could be recovered.

As to the question whether or not such satisfactory results as above of the operation can remain permanently, the writer has no ample experience. The second patient had an attack after his return-home. How is this unexpected renewed attack explainable? At first the sequel depends upon the dexterity by the operation, that is to say, a suitable window must be settled on the saccus endolymphaticus and the window must be kept open as to avoid the increase of the endolabyrinthine pressure. Secondary, the attack may be concidered as a mount in the course of recovery as after-shock of earthquake. And in the third place, if one makes a wrong diagnosis of virtigo, it will be naturally unable to obtain complete recovery through this operation. However, he became very vigorous after that and intended to return to his former occupation, when the writer visited him in Jan. of this year (one year after the operation).

The fourth patient was relieved from her one year's sick-bed by the operation. After return-home she could carry a pail of water over 120 yards without suffering any ill effects and she asked the writer, if she might resume her work as a teacher. She taught school, but had afterwards an attack and since then has been unwell. Concerning the cause of this new attack, it will be necessary to trace the further course of the illness.

When only the labyrinth of one side is affected, we can expect, that the disease will be recovered completely through the operalion, but if the involvement of the labyrinth began on both sides from the onset, what would be the course after the operation? A possibility is that the same symptoms occur in the non-operated side after healing of the operated side.

However it may be, the writer made opening of the saccus endolymphaticus. In consequence all the symptoms disappeared at least immediately after the operation. And what establishes a connection between these two facts was simple allowing of the endolabyrinthine fluid to flow out. Here, the most important of the conditions within the labyrinth that have been altered by the operation legitimately considered to be the endolabyrinthine pressure and from the effective results the increase of the pressure might be regarded as the only or chief cause of this disease.

Conclusion.

5 patients who suffered from attacks of virtigo, were subjected to the Portmann's operation.

They suffered from virtigo and confined in bed before the operation and the operation made them possible to escape from so-called status

labyrinthicus and to return not only to their healthy daily life, but to their former occupation.

This one group of labyrinth diseases, on which the dscharging treatment of labyrinthine fluid has a therapeutic effect, shows the following symptoms;

It begins usually with tinnitus of one side and before long gives rise suddenly to attacks of virtigo without any suspicious cause. At about the same time hearing begins to diminish. The deafness increases progressively. The virtigo is often severe and associated with sense of movement either of himself or of external objects. When attacks are severe, nausea and vomitting also occur. These accesses appear often paroxysmally. The duration and the severity of attacks are various. There are several minor accesses before and after a mojor attack and even between two attacks the patients are often in status labyrinthicus and suffer from dizziness and heaviness of the head on the affected side.

In view of the fact that these symptoms can be relieved relatively easily by means of draining the endolymph as described above, the wirter came to the conclusion, that the occurrence of symptoms is due to the increase of the endolabyrinthine pressure. Although the access of virtigo is one of the important symptoms of this group of labyrinth diseases and affords often a clue to find the diseases clinicaly, the entity of the disease behind the symptoms must be an increase of the endolabyrinthine pressure or at least a process accompanied by it, proceeding continuously no matter what the clinical manifestations.

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1) Portmann, J. of Laryng. 1927, 42, 809.

labyrinthion and to return not only to their healthy daily life, but to their former occupation.

The one group of labyrinth diseases, on which the destroying treatment of labyrinthine fluid has a therapeutic effect, shows the following symptoms:

It begins usually with tinnitus of one side and before long gives rise suddenly to attacks of vertigo without any suspicious cause. At about the same time hearing begins to diminish. The deafness increases progressively. The vertigo is often severe and associated with nausea of more or less either of himself or of external objects. When attacks are severe, nausea and vomiting also occur. These symptoms often paroxysmally. The duration and the severity of attacks are various. There are several minor accesses before and after a major attack and even between two attacks the patients are often ill from labyrinthitis and suffer from dizziness and heaviness of the head on the affected side.

In view of the fact that these symptoms can be removed relatively easily by means of draining the endolymph as described above, the writer came to the conclusion, that the occurrence of symptoms is due to the increase of the endolabyrinthine pressure. Although the access of vertigo is one of the important symptoms of this group of labyrinth disease and affords often a clue to find the disease clinically, the entity of the disease behind the symptoms must be an increase of the endolabyrinthine pressure, what ist a process accompanied by a proceeding continuously no matter what the clinical manifestations.

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